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<p>(54) Title: A METHOD FOR PROMOTING TISSUE REPAIR</p> <p>(57) Abstract</p> <p>A combination of a first compound of an oligo- or polysaccharide containing aminosugar units and a second compound of a sulfated mono-, di- or oligosaccharide as an agent for the enhancement of the healing of wounds in collagen containing tissues, including skin and bone and in mucosa. The first compound may be, e.g., chitosan, chitosans obtained by deacetylated of chitin to various degrees of deacetylation, chitosan derivatives, glycosaminoglycans including chondroitin, chondroitin sulfate, hyaluronic acid, dermatan sulfate and keratan sulfate; aminated dextrans including DEAE-dextran; aminated starch, aminated glycogen, aminated cellulose, aminated pectin, heparin, and salts, complexes, derivatives and mixtures thereof, and the second compound may be a disaccharide such as, e.g., sucrose, a sucrose derivative or a complex or a salt thereof, wherein the disaccharide is at least tetrasulfated. A very interesting combination is a combination of chitosan as the first compound and sucrose octasulfate as the second compound. Also disclosed is the use of such compounds as medicaments, pharmaceutical compositions containing such combinations and a method for the preparation of such combinations.</p>			

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A METHOD FOR PROMOTING TISSUE REPAIR

The present invention relates to a combination of a first compound of an oligo- or polysaccharide containing aminosugar units and a second compound of a sulfated mono-, di- or oligosaccharide, and most preferably to a combination of chitosan and sucrose octasulfate, as an agent for the
5 enhancement of the healing of wounds in collagen containing tissues, including skin and bone and in mucosa.

Wounds and/or ulcers are normally found on the skin or on mucosal surfaces. In the present context the term "skin" relates to the outermost surface of the body of an animal such as a human and embraces intact or almost intact skin as well as injured skin surfaces. The term
10 "mucosa" relates to undamaged or damaged mucosa of an animal such as a human and may be the oral, buccal, aural, nasal, lung, gastrointestinal, vaginal, or rectal mucosa.

The term "skin" is used in a very broad sense embracing the epidermal layer of the skin and - in those cases where the skin surface is more or less injured - also the dermal layer of the skin. Apart from the stratum corneum, the epidermal layer of the skin is the outer (epithelial) layer
15 and the deeper connective tissue layer of the skin is called the dermis. The skin may have a thick or a thin epidermis and is therefore often classified as thick or thin skin. In the present context, the term "skin" embraces thick skin as well as thin skin.

Thick skin is found on the palms of the hands and the soles of the feet, whereas thin skin covers the remainder of the body. The skin on the palms of the hands and the soles of the feet has a
20 thick epidermis with a particularly thick layer of keratin on its outer surface. The skin covering the remainder of the body has a relatively thin epidermis and the outer keratinized layer of the epidermis is relatively thin.

The skin forms a barrier between the body and the environment and one of the most important functions is to protect the body from invasion by potentially hazardous materials and organisms.
25 The skin consists of two layers of completely different kinds of tissue that are attached to one other over their whole extent. The outer layer contains stratified squamous keratinizing epithelium. It does not contain any blood vessels so it must be nourished via tissue fluid from a second and deeper layer of the skin. This second layer contains irregularly arranged connective tissue containing blood vessels. The outermost layer of the skin is the so-called stratum
30 corneum. Then there is a viable layer called the epidermis and the papillary dermis layer.

The epidermis and particularly its layer of keratin is a barrier to disease organisms. Keratin is nearly water-proof and makes it possible to have a bath without swelling or shrinking of the

skin. The epidermis contains cells producing melanin and is therefore able to protect the body from harmful effects of e.g. ultraviolet light. Furthermore, the skin serves as a temperature regulator and contains nerve endings responsible for picking up stimuli that evoke many different kinds of sensation during consciousness (e.g. touch, pressure, heat, cold and pain).

- 5 Hence the skin is of great importance in permitting man to adjust to his environment.

Since the skin is the most exposed part of the body, it is particularly susceptible to various kinds of injuries such as, e.g., ruptures, cuts, abrasions, burns and frostbites or injuries arising from various diseases. Furthermore, much skin is often destroyed in accidents. However, due to the important barrier and physiologic function of the skin, the integrity of the skin is important to

- 10 the well-being of the individual, and any breach or rupture represents a threat that must be met by the body in order to protect its continued existence.

Apart from injuries on the skin, injuries may also be present in all kinds of tissues and injuries like abscesses and internal ulcers are also treatable with a combination according to the invention. These injuries can have a wide range of severity and can have numerous internal as

- 15 well as external causes. Probably the most frequent tissue injury is skin wounds due to simple external trauma. A tissue injury on a body surface caused by a disease is typically called an ulcer irrespective of its presence on the skin, in the gastrointestinal tract or any other mucosal surface (cf. below).

- A tissue injury starts a cascade of reactions aimed at minimising the injury and preparing for
20 tissue repair. Dependent on the type of tissue involved and the nature of the injury, different processes are more or less prominent. A process that aims at minimizing the injury is typically an inflammatory reaction, which even to some extent by itself can cause tissue injury. The transition from an inflammatory process to a tissue repair process is gradual and both processes can occur at the same time. The tissue repair process will only start if the tissue injury process
25 is successfully stopped either by a physiologic process alone or in combination with a specific therapy. Furthermore, conditions in the tissue must allow that the repair process can occur. For instance in crural leg ulcers a certain minimal blood supply is required for the healing process to be possible. When these conditions are met, the tissue repair process can start.

- In the early stage of the tissue repair, one process is almost always involved and that is the
30 formation of a transient connective tissue in the area of tissue injury. This process starts by forming a new extracellular collagen matrix by fibroblast. This new extracellular collagen matrix is then the support for a connective tissue during the final healing process. The final healing is in most tissues a scar formation containing connective tissue. In tissues which have regenerative properties, such as, e.g., skin and bone, the final healing includes regeneration of the original

tissue. This regenerated tissue has frequently also some scar characteristics, e.g. a thickening of a healed bone fracture.

As almost all tissue repair processes include the early connective tissue formation, a stimulation of this and the subsequent processes are contemplated to improve tissue healing. In principle all
5 tissue repair can benefit from a stimulation but the clinical impact of a stimulation of tissue healing will differ depending on the type of tissue injury and the subsequent healing process. The value of a tissue healing stimulation is larger in conditions where the healing time is prolonged. A clinically important example of such a condition is leg ulcers.

In the following, the healing process is exemplified by description of the process for a wound
10 present on the skin.

Under normal circumstances, the body provides mechanisms for healing injured skin in order to restore the integrity of the skin barrier. The repair process for even minor ruptures or wounds may take a period of time extending from hours and days to weeks. However, in ulceration, the healing may persist for extended period of time, i.e. months or even years. Thus, failure to heal
15 of cutaneous ulcers is a serious clinical problem which is accompanied by significant financial and emotional costs.

The healing process is a complex and well orchestrated physiological process that involves migration, proliferation and differentiation of a variety of cell types as well as synthesis of matrix components. Normally, the healing process comprises of three phases:

20 i) Haemostasis and inflammation

When platelets are present outside the circulatory system and exposed to thrombin and collagen, they become activated and they aggregate. Thus, platelets initiate the repair process by aggregating and forming a temporary plug to ensure haemostasis and prevent invasion from bacteria. The activated platelets initiate the coagulation system and release growth factors like
25 platelet-derived growth factor (PDGF) and epidermal growth factor (EGF).

The first cells to invade the wound area are neutrophils followed by monocytes which are activated by macrophages.

The major role of neutrophils appears to be clearing the wound of or defending the wound against contaminating bacteria and to improve the healing of the wound by removing dead cells
30 and platelets. The infiltration of neutrophils ceases within about the first 48 hours provided that

- no bacterial contamination is present in the wound. Excess neutrophils are phagocytosed by tissue macrophages recruited from the circulating pool of blood-borne monocytes. Macrophages are believed to be essential for efficient wound healing in that they also are responsible for phagocytosis of pathogenic organisms and a clearing up of tissue debris. Furthermore, they 5 release numerous factors involved in subsequent events of the healing process. The macrophages attack fibroblasts which start the production of collagen.

ii) Granulation tissue formation and re-epithelialization

- Within 48 hours after wounding, fibroblasts begin to proliferate and migrate into the wound space from the connective tissue at the wound edge. The fibroblasts produce collagens and 10 glycosaminoglycans and inter alia low oxygen tension at the wound stimulates proliferation of endothelial cells. The endothelial cells give rise to the formation of a new capillary network.

- Collagenases and plasminogen activators are secreted from keratinocytes. If the wound is left undisturbed and well-nourished with oxygen and nutrients, keratinocytes will migrate over the wound. Keratinocytes are believed only to migrate over viable tissue and, accordingly, the 15 keratinocytes migrate into the area below the dead tissue and the crust of the wound.

The wound area is further decreased by contraction.

iii) Dermal remodelling

As soon as the re-epithelialization is completed the remodelling of the tissue begins. This phase, which runs for several years, restores the strength to the wounded tissue.

- 20 All of the above-mentioned healing processes take considerable time. The rate of healing is influenced by the wound's freedom from infection, the general health of the individual, presence of foreign bodies, etc. Some pathologic conditions like infection, maceration, drying out, generally bad health and malnutrition can lead to formation of a chronic ulcer.

- 25 Until at least superficial healing has occurred, the individual remains at risk of continued or new infection. Therefore, the quicker the wound can heal, the sooner the risk is removed.

Thus, any procedure that can influence the rate of wound healing or favourably influence the healing of wounds is of great value.

Numerous treatments are currently used to create an optimal environment for the wound healing process. However, to our knowledge none of these treatments have been designed to directly stimulate the wound repair process. It has previously been suggested that growth factors like epidermal growth factor (EGF), transforming growth factor- α (TGF- α), platelet derived growth factor (PDGF), fibroblast growth factors (FGFs) including acidic fibroblast growth factor (α -FGF) and basic fibroblast growth factor (β -FGF), transforming growth factor- β (TGF- β) and insulin like growth factors (IGF-1 and IGF-2) are conductors of the wound healing process.

The present invention is based on a novel approach to wound healing, namely an approach involving the use of a wound healing composition comprising as an active wound healing substance a combination of two substances, where the first substance in the combination inter alia has a function of immobilizing the combination in the wound area in order to obtain an enhanced and/or sustained or prolonged effect, and the second substance is able to bind and/or activate and/or stabilize growth factors involved in the healing process. As will be explained in detail below, a particularly interesting combination according to the present invention is a combination between chitosan and sucrose octasulfate. Although both substances are known as promoters in the wound healing process, the combination according to the present invention has an unexpected useful, superior wound healing effect compared to the wound healing effect obtained by use of the individual substances alone or in a physical blend. Furthermore, the use of a combination according to the invention with the purpose of healing wounds is very advantageous; thus, in the case of chitosan-sucrose octasulfate the chitosan aids in immobilizing sucrose octasulfate (i.e. a wound healing agent) site specifically at/on the location at/on which the wound healing agent is to exert its effect. Moreover, the combination of e.g. chitosan-sucrose octasulfate has a water solubility which is much lower than that of sucrose octasulfate by itself. If sucrose octasulfate is applied onto a wound, the pronounced water solubility of sucrose octasulfate will result in a very fast transport of sucrose octasulfate away from the site of action and, thus markedly reduce the presence of the active substance and, consequently, markedly reduce the time period during which the active substance can exert an effect. The overall effect is therefore a short lasting effect of sucrose octasulfate and, accordingly, sucrose octasulfate has to be applied onto the wound very frequent (e.g. 3-7 times daily) in order to achieve the desired effect.

When treating many kinds of wounds specific precautions have to be taken into considerations, such as, e.g., sterility considerations, contamination problems, correct application of bandages/dressings etc. which normally require that the treatment/application is performed by well-educated nurses or the like, i.e. wound treatment becomes a very expensive operation when the wound healing agent is to be applied several times daily. A desired reduction in the costs involved in wound healing treatment is therefore obtainable when the application frequency can

be reduced. By using a combination of e.g. chitosan and sucrose octasulfate it is possible to take advantage of the low water solubility of the combination, i.e. the combination will stay on/at the application site and at that site slowly release sucrose octasulfate as a wound healing agent. The solubility of the sucrose octasulfate released at the desired site (wound) has been found to be of 5 the same order of magnitude as that of sucrose octasulfate in itself. In addition to the beneficial wound healing effect of sucrose octasulfate, chitosan in itself has also been found to have a positive impact on wound healing.

The present inventors have found that although sucrose octasulfate and chitosan may react in situ after application of the two individual substances in vivo on a wound and form a 10 combination of chitosan and sucrose octasulfate (this reaction takes place when the individual substances are applied in the form of powders, two different gels or in the form of solutions or two separate formulations) such a reaction proceeds in an uncontrollable manner, i.e. resulting in a combination having an uncontrollable and variable content of chitosan and sucrose octasulfate, respectively. Therefore, even if chitosan and sucrose sulfate to a minor degree react 15 with each other and form the desired combination in situ in/on a wound after application of the individual substances, the reaction is so uncontrollable and unreproducible that the product obtained is too undefined for medical purposes.

The therapeutic and/or prophylactic activity of a combination according to the invention may of course be evidenced by in vivo tests using experimental animals or humans. However, an 20 indication of the efficacy and/or activity of a combination according to the invention can be obtained by performing relatively simple in vitro tests such as, e.g., tests involving cell cultures.

Numerous in vivo as well as in vitro test can be employed for the determination of whether a combination according to the invention has a beneficial effect on the healing process. Reference is made to the following relevant tests but other tests may also prove suitable:

- 25 i) a test assessing epidermal regeneration (cf. A. Fourtanier et al., Br. J. of Dermatology
 (1984) III, Suppl. 27, 174-177,

 ii) a test involving a diabetic mouse wound healing model (cf. B. Matuszewska et al., Pharm.
 Res. (1994), Vol. 11, No. 4, 65-71),

 iii) a test model for wound healing (cf. H.P. Dinger & H. Redl, Wiener Klinische
 Wochenschrift (1987), Vol. 99, No. 14, 497-501),

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- iv) a test for the regeneration of full-thickness wounds (cf. M.J.A. van Luyn et al., J. Biomed. Mat. Res. (1995), No. 29, 1425-1436),
 - v) a test involving cell cultures and measurement of collagen synthesis (cf. T. Kato & P.M. Royce, Biomed. Res. (1995), Vol. 16, No. 3, 191-198),
- 5 vi) a test involving formation of e.g. a partial thickness excisional wound or a second degree burn wound (cf. P.M. Mertz et al., Cosmetics & Toiletries (1992), Vol. 107, 43-44),
- vii) a wound healing model in a scald burn injury pig model (cf. J.A. Bauer et al., Lipid Mediators Immunol. (1987), Vol. 139, 519-522),
 - viii) an organotypic in vitro model (cf. J.A. Garlick and L.B. Taichman, J. Invest. Dermatol. 10 (1994), Vol. 103, 554-559), and
- 10 ix) an in vitro model involving skin fibroblasts (cf. L.W. Adams & G.C. Priestley, Arch. Dermatol. Res. (1988), Vol. 280, 114-118).
- 15 x) a test identifying wound healing associated indicators such as, e.g., indicators of pH, partial pressure O₂, temperature, radical mechanisms or biotechnological assays, e.g. indicating a formation of collagen,
- xi) an in vitro model for wound healing wherein pieces of human skin is placed in a cell growth medium containing fetal calf serum (FCS); after fixation and staining the reepithelialisation is studied by means of a light microscope,
 - xii) in vitro tests designed for evaluation of components which impart the healing process (e.g. neutrophils, macrophages, fibroblasts, growth factors, collagen, collagenase, cell proliferation, and epidermal cell migration, i.e. epiboly) (e.g. cf. S.M. Vijayasingham et al., Br. J. Dermatol. (1991), Vol. 125, 136-139, and/or S. Regauer & C.C. Compton, J. Invest. Dermatol. (1990), Vol. 95, 341-346),
- 20 xiii) a test involving basic fibroblast growth factor stimulation of epidermal wound healing in e.g. pigs (cf. P.A. Hebda et al., J. Invest. Dermatol. (1990), Vol. 95, 626-631),
- 25 xiv) an in vitro test involving growing of a cell culture for the determination of the influence of the extracellular matrix on fibroblast responsiveness (cf. P.G. Genever, Br. J. Dermatol. (1995), Vol. 133, 231-235), and

- xv) a test involving histological examination of the tissue or cell culture.

In a first aspect the invention relates to a combination of a first compound of an oligo- or polysaccharide containing aminosugar units and a second compound of a sulfated mono-, di- or oligosaccharide. In the present context the term "oligosaccharide" is intended to mean a

- 5 carbohydrate containing from two and up to ten simple sugar (i.e. monosaccharide sugar) units linked together. Beyond ten they are denoted polysaccharides. Within the scope of the present invention is not combinations of e.g. aminosugar containing polysaccharide-sulfated polysaccharide compounds and therefore, the disclosures in EP-A-0 427 190 and EP-A-0 454 044 (both in the name of Hoechst Aktiengesellschaft) concerning polyelectrolytic complexes of e.g.
10 chitosan and dextransulfate or xylanpolysulfate are clearly without the scope of the present invention as the complexes are composed of two polysaccharide substances.

The combination of the first and second substance is not merely a physical blend of the two substances since some kind of binding forces are involved in the formation of the combination. Most likely, the combination is held together by ionic binding between the amino groups of the

- 15 first substance and the sulfated groups of the second substance, but other binding forces may also have an impact. Thus, the combination may be a salt, a complex such as, e.g., an inclusion complex, or the substances may be held together in a polymeric network or as a mechanical inclusion, or even - although more unlikely - the substances may be covalently bound together in the combination. Further experiments are presently ongoing in order to determine the structure
20 of the combination.

The fact that the combination according to the invention is not merely a physical blend is evidenced by subjecting the combination to at least one of the tests described in the following and based on the results thereof, the combination must at least fulfil one of the following criteria:

- 25 i) a thermogram obtained by subjecting the combination to Differential Scanning Calorimetry must be significantly different from thermograms obtained by subjecting the first compound, the second compound and a physical blend thereof, respectively, to Differential Scanning Calorimetry;
- ii) an IR-spectrum of the combination must be different from the spectre of the individual
30 first and second compounds and from a physical blend of the individual first and second compounds;

- iii) an NMR-spectrum of the combination must be different from the spectre of the individual first and second compounds and from a physical blend of the individual first and second compounds;
- iv) an X-ray diffraction spectrum of the combination must be different from the spectre of the individual first and second compounds and from a physical blend of the individual first and second compounds;
- 5 v) the combination must have a solubility in 1% acetic acid at room temperature of at the most about 5% w/w such as, e.g., at the most about 4%, 3%, 2%, 1%, 0.5%, 0.25%, 0.1%, 0.05%, 0.01%, 0.005% or 0.001% w/w; and
- 10 vi) the combination must have a solubility in water at room temperature of at the most about 5% w/w such as, e.g., at the most about 4%, 3%, 2%, 1%, 0.5%, 0.25%, 0.1%, 0.05%, 0.01%, 0.005% or 0.001% w/w.

The experimental details with respect to the tests described above are found in the experimental section herein.

- 15 Other tests may of course also be relevant such as, e.g., a test involving mass spectroscopy, or an in vitro model for testing the properties of the combination. Relevant in vitro models are described above. Such tests may either be employed with a view to determine any differences in the properties of a combination according to the invention compared with the properties of the individual compounds and a physical blend thereof, or they may be employed as in vitro models
- 20 for the therapeutic activity/effect of a combination according to the invention.

- As mentioned above, the first compound is an oligo- or polysaccharide containing aminosugar units. In the present context, an aminosugar unit is a sugar unit wherein at least one of the hydroxy groups available in the sugar unit has been substituted by an amino group or an alkanoylated amino group such as an acetylated amino group. Apart from the aminosugar units,
- 25 the polysaccharide may contain unsubstituted sugar units or sugar units substituted with e.g. alkoxy (such as 2,3-dimethylglucose) or acyloxy.

In particular, in embodiments of the invention, at the most about 30% such as, e.g., at the most about 25%, about 10% or about 5% of the aminosugar units are alkanoylated. In some cases, it may also prove suitable that none of the aminosugar unit are alkanoylated.

The first compound preferably has a degree of amination in the range of 0.1-4, such as, e.g. in the range of 0.1-3 or 0.1-2, in particular in the range of 0.5-1.5 such as about 1, the degree of amination being defined as the average number of amino groups or alkanoylated amino groups per saccharide unit.

- 5 In certain embodiments according to the invention, the first compound has a degree of amination of about 1. Such a first compound is, e.g., a first compound wherein each sugar unit contains an amine or an alkanoylated amine group.

- The individual sugar units of the first compound may be the same or different and are pentoses or hexoses. Examples of pentoses include fructofuranose. The hexoses are selected from the
- 10 group consisting of glucose, mannose, galactose, xylose and ribose. Furthermore, units like N-acetyl-D-glucosamine and/or N-acetylmuramic acid or the deacetylated compounds thereof may be incorporated in a first compound of the invention.

- Specific examples of interesting first compounds are compounds selected from the group consisting of chitosan, chitosans obtained by deacetylation of chitin to various degrees of
- 15 deacetylation, chitosan derivatives, glycosaminoglycans including chondroitin, chondroitin sulfate, hyaluronic acid, dermatan sulfate and keratan sulfate; aminated dextrans including DEAE-dextran; aminated starch, aminated glycogen, aminated cellulose, aminated pectin, heparin, and salts, complexes, derivatives and mixtures thereof.

- In particular chitosan and chitosan derivatives are promising candidates as a first compound.
- 20 Water-soluble chitosan has been described as an agent in the treatment of wounds; the chitosan is said to prevent the formation of fibrin strands (US Patent No. 4,532,134). Such a prevention of the formation of fibrin strands is believed to promote the proliferation of fibroblasts and the synthesis of collagen thereby allowing the promotion of normal tissue regeneration.

- Chitosan has also been applied in the development of delivery systems as a means for obtaining
- 25 controlled release of drugs (see e.g. WO 96/05810). Furthermore, chitosans have been found to have mucoadhesive properties (see e.g. EP 514 008, C-M. Lehr et al., "In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers, from C-M. Lehr's Ph.D. thesis, 1991, Leiden University, the Netherlands) and the use of chitosan as an enhancer has been suggested.
 - 30 Chitosans are considered as biocompatible macromolecules due to assumed low toxicity and biodegradability. They are degraded by lysozymes and related enzymes, such as N-acetyl-D-glucosaminidases. The lysozymic degradation of chitosan is believed to increase with the degree

of N-acetylation (see e.g. Aiba, Int. J. Biol. Macromol., 14, 1992, 225-228) and this property may influence the release rate of the second compound in a combination according to the invention (see below). Accordingly, chitosan for therapeutic use must be regarded as a safe substance.

Chitosan is a linear 1,4-bound polysaccharide built up from β -glucosamine entities. The chitosan
5 is manufactured by N-deacetylation of chitin which is a naturally occurring polymer forming the shell of inter alia insects and crayfish. Commercial chitin is recovered from crab and shrimp shell which are waste products from the fishing industry. It is possible to manufacture chitosans of varying degrees of N-acetylation by controlling the reaction conditions (e.g. controlling the alkaline treatment of chitin). Furthermore, chitosan may be manufactured with different
10 molecular weights (Antonsen et al., Carbohydrate Polymers, 22, 1993, 193-201). Different molecular weights of chitosan can be obtained by controlling the degradation of a high molecular weight chitosan in e.g. 1 N hydrochloric acid or by subjecting the chitosan to a controlled enzymatic degradation. When chitin is treated with alkali such as sodium hydroxide, N-deacetylation takes place and the acetamido groups are converted into amino groups to form
15 chitosan. Thus, in qualities of chitosan having a number of acetamido groups left, the aminosugar in the first compound of a combination according to the invention is totally or partly alkanoylated with acetamido groups or even in some cases all parent acetamido groups have been deacetylated to amino groups.

It is believed that the degree of acetylation as well as the molecular weight of a chitosan have
20 some impact on the properties of the combination formed. Thus, it is believed that the in vivo release of the second compound from a combination (where the first compound is a chitosan) is subjected to at least one mechanism involving the degradation of the chitosan by an enzymatic process e.g. involving enzymes such as, e.g., lysozyme and proteases (present in wounds/ulcers), hyaluronidases, chitonases, metalloproteinases, collagenases, elastases and/or a combination
25 thereof. As mentioned above, the lysozymic degradation of chitosan increases with the degree of N-acetylation and, accordingly, the release rate of the second compound in the combination may increase with the degree of N-acetylation. Another release mechanism operating either alone or in combination with the above-mentioned mechanism may be based on a change in ionic strength; the higher the ionic strength is in the environment the faster the release is expected to
30 take place due to the theory that the combination may be held together inter alia by ionic bondings and/or by inclusion. Without being bound to any theory the above-mentioned two release mechanisms seem to be most important. Furthermore, these release mechanisms seem to support the indication i) that the molecular weight of the chitosan comprised in the combination has an impact on the release rate (i.e. mainly for the release mechanism involving
35 enzymatic degradation), and ii) that the degree of deacetylation of the chitosan comprised in the combination may have an impact on the release rate (i.e. probably for the release mechanism

involving a change in ionic strength of the release medium). It is believed that the molecular weight as well as the degree of deacetylation also are important determinants for i) the biodegradability of a chitosan, ii) the toxicity, iii) the degree of mucoadhesiveness, and iv) the enhancer effect.

- 5 Apart from the above-mentioned theory with respect to the release of the second compound (such as, e.g., sucrose octasulfate), the first and/or second compound or even the combination by itself may exert its/their effect in undissolved stage e.g. in the form of fragments of molecules extending out from the particle surface. Furthermore, fragments of dissolved second compound such as, e.g., sucrose octasulfate, first compound such as, e.g., chitosan and/or the combination
10 between the first and the second compound such as, e.g., chitosan-sucrose octasulfate, respectively, may also be effective with respect to wound healing. Another possibility is that sucrose octasulfate (representative of a second compound) is capable of exerting its effect when at least some of the chitosan (representative of a first compound) is degraded to smaller fragments; an ion-exchange process may be involved in the release/availability of sucrose
15 octasulfate.

Thus, in particular preferred embodiments of the invention, the first compound is a chitosan, especially a chitosan which has a molecular weight in a range of about 3,000 to about 1,500,000 daltons. With respect to the degree of deacetylation, the chitosan notably has a degree of deacetylation of at the most 100% such as at the most 99%,
20 95%, 90%, 85%, or 80%, or expressed in another way the chitosan has a degree of deacetylation in a range of about 10-90% such as about 20-85%, about 30-80%, about 40-75%, about 50-75%, about 60-85%, about 75-85%, or about 80-90%.

The concentration of chitosan in the combination is in a range of from about 5 to about 99.99% w/w such as, e.g. from about 10-80% w/w, about 20-75% w/w, about 30-70% w/w or about 40-25 70% w/w based on the total combination.

As thoroughly discussed above, the first compound of a combination according to the invention is a polysaccharide containing aminosugar units. However, other first compounds may also prove suitable for use in a combination according to the invention provided that such first compounds are capable of reacting with a second compound (see below for details) to form a combination
30 which is not merely a physical blend of the two individual compounds (evidenced as described above), which is capable of releasing the second compound e.g. in wounded tissue, and which is biocompatible and biodegradable. A proviso for such other kinds of suitable first compounds may be found in the group consisting of e.g. natural and synthetic polymers containing at least one amino group.

The second compound of the combination is a polysulfated mono-, di- or oligosaccharide. The monosaccharide may be, e.g., fructofuranose, glucose, mannose, galactose or ribose. Particularly promising second compounds are polysulfated disaccharides, such as compounds based on, e.g., sucrose, a sucrose derivative or a complex or salt thereof.

- 5 The saccharide is polysulfated or even persulfated, which means that at least two and preferably more (such as all) hydroxy groups are sulfated. In the case where the polysulfated saccharide is sucrose, the sucrose is at least tetrasulfated, preferably the sucrose is sucrose octasulfate or a salt or a complex thereof including the sodium salt thereof.

- 10 The sulfated saccharide may be complexed with or form a salt with an alkali metal (e.g. sodium or potassium), an alkaline earth metal (e.g. magnesium or barium) or another metal (e.g. aluminum, etc.)

- When sucrose octasulfate is employed as a second compound in the combination, the content thereof in the combination is in a range of 0.01-95% w/w such as, e.g., in a range of about 20-90% w/w, 25-80% w/w, 30-70% w/w or 30-60% w/w based on the weight of the total
15 combination.

In a particularly important embodiment, the combination is formed between a chitosan as the first compound and a sucrose octasulfate as the second compound.

- The chitosan may be chitosan polymer having a molecular weight in a range of from 10 kdalton to 1500 kdalton and a degree of deacetylation in a range of at the most 100%. Chitosan is
20 presently available in qualities having a mean molecular weight in a range of from about 10 kdalton to about 1000 kdalton and a degree of deacetylation in a range of from about 75% to about 85%, but chitosans of other mean molecular weights and having other degrees of deacetylation are obtainable by employment of methods well known to a person skilled in the art (cf. above). Examples of chitosans are chitosan, Wella "low viscosity", C., Wella "high viscosity",
25 C., Dr. Knapzyk, Daichitosan H, Daichitosan VH, SeaCure 240, SeaCure 210, Chitosan (Sigma), Polycarbophil/daichitosan VH blend, Protasan CL 210, Protasan G210, and Protasan G110. Furthermore, chitosans having a mean molecular weight of about 70,000-500,000 such as about 250,000-500,000 and a degree of N-acetylation of about 10-50% such as about 20-40% may be interesting candidates as first compounds in a combination according to the invention. The
30 chitosan may be in the form of a chitosan base or in the form of a salt such as, e.g., a glutamate, a lactate, or a hydrochloride salt. Mixtures of chitosan base and one or more chitosan salts are within the scope of the present invention as well as mixtures of chitosans having different mean molecular weights and/or degrees of deacetylation.

An interesting example of a combination according to the invention is a combination wherein the first compound is a chitosan having a molecular weight in a range from about 5,000 to about 100,000 dalton and the second compound is a sucrose octasulfate.

As mentioned above the combination is an active substance for use in medical treatment. The
5 combination may be used in the treatment or prevention of any relevant conditions, especially in conditions involving tissue repair such as in the healing of wounds. Furthermore, when the combination is a combination of a chitosan and a sucrose octasulfate, the combination may be used in the treatment or prevention of any condition or disease where a sucrose octasulfate has a beneficial effect. In this connection, a sucrose octasulfate includes sodium or potassium sucrose
10 octasulfate or the aluminium complex of sucrose octasulfate (known as sucralfate) or a combination thereof. Thus, the combination may also be used for the treatment or prevention of inflammatory conditions or for any condition which respond positively to a treatment with sucrose octasulfate.

A combination of chitosan as a first compound and a sucrose octasulfate as a second compound
15 is believed to have properties which are highly valuable in connection with wound treatment. Thus, chitosan is a biocompatible and relatively slowly biodegradable compound and sucrose octasulfate is also biodegradable. A combination of these two compounds is therefore believed also to be biodegradable. As mentioned above, chitosan is degraded in vivo by enzymes, e.g. lysozyme, proteases, and/or other enzymes present in exudates, and - as explained in the
20 experimental section herein - we have found that the combination releases sucrose octasulfate in vitro when the combination is subjected to lysozymic degradation in an aqueous medium.

Lysozyme is present in a number of body fluids and is released by leukocytes and other cells normally present in wounds and other tissues where tissue repair is ongoing. A working theory for a combination of chitosan and sucrose octasulfate is that when the combination is applied to
25 a wound or a tissue which is to be repaired, then the lysozyme and/or protease present in the wound or tissue will degrade the combination, thereby releasing the sucrose octasulfate from the combination. As a result, a combination will have a prolonged effect and less frequent administration is required compared to administration of sucrose octasulfate alone. Frequent topical administration to a wound is generally inconvenient for the individual; wound dressings
30 are normally changed every other day or at the most once daily which limits the frequency of possible administration to wounds. With respect to internal wounds arising from surgery, the frequency of administration is generally very limited and normally only one administration is possible.

As indicated above, the presently most important use of a combination according to the invention is the use as a wound healing agent, i.e. an agent which accelerates, stimulates or promotes healing of dermal or mucosal wounds. Yet another important use, is the use as a tissue repair agent. The term "wound" used in the present context denotes any wound (see below for a 5 classification of wounds) and at any particular stage in the healing process including the stage before any healing has initiated.

A composition according to the invention will typically stabilise and/or stimulate fibroblast growth factors (FGF), the formation of collagen or the healing of wounds in vitro when subjected to relevant test like the tests described herein.

10 In general, the term "wound" denotes a bodily injury caused by physical means, with disruption of the normal continuity of structures. Examples of wounds are, e.g., contused wounds, incised wounds, penetrating wounds, perforating wounds, puncture wounds, subcutaneous wounds, etc. The term "ulcer" normally denotes a local defect, or excavation, of the surface of an organ or tissue, which is produced by the sloughing of inflammatory necrotic tissue. Examples of ulcers 15 are, e.g., peptic ulcer, duodenal ulcer, gastric ulcer, gouty ulcer, diabetic ulcer, hypertensive ischemic ulcer, stasis ulcer, sublingual ulcer, submucous ulcer, symptomatic ulcer, trophic ulcer, tropical ulcer, venereal ulcer, etc. However, there is often a certain overlap between the use of the terms "wound" and "ulcer" and, furthermore the terms are often used at random. Therefore, in the present context the terms "wound" and "ulcer" are indiscriminately used unless otherwise 20 indicated.

In general wounds may be classified as follows:

- i) Mechanical injuries (e.g. abrasion, lacerations, penetrating wounds, bites, and surgical wounds),
- ii) Burns and chemical injuries [e.g. superficial burns (first degree), deep dermal burns 25 (second degree), and full thickness (third degree)],
- iii) Chronic ulcerative wounds
 - a) decubitus ulcer also called bed or pressure sores
 - b) leg ulcers (venous, ischaemic or traumatic)
 - c) ulcers associated with certain systemic infections

- d) ulcers resulting from radiotherapy
- e) ulcers resulting from malignant diseases.

In general wounds may also be classified by appearance as follows:

- i) black and necrotic wound covered with a hard, dry black necrotic layer (e.g. small and 5 superficial wounds or extensive and deep wounds)
- ii) yellow and sloughy wounds covered with (or filled) with a soft yellow slough (e.g. small and dry wound, small and moist wounds, and large deep cavities containing semi-liquid necrotic material)
- iii) clean wounds with significant tissue loss (granulating wounds such as, e.g., clean surgical 10 wounds with significant tissue loss, chronic wounds with low to moderate exudate, chronic open wounds with moderate to high exudate, and chronic flask-shaped wounds)
- iv) clean and superficial wounds (epithelialising wounds such as, e.g., clean, low exudate 15 wounds, and clean wounds with medium to high exudate (e.g. burns, donor sites))
- v) clinically infected wounds (such as, e.g., extensive or heavily exuding wounds, small 20 cavities or craters, and shallow open wounds)
- vi) malodorous wounds (e.g. infected pressure sores, fungating carcinomas, etc.)

Wounds/ulcers may be of a chronic or acute character. Vascular, diabetic and decubitus wounds belong to the group of chronic wounds, whereas e.g. burns belong to the group of acute wounds. As mentioned above, wounds may be a result of a physical injury, but the presence of

- 20 wounds/ulcers may also be an indication of an underlying disease or disturbance such as, e.g., diabetes, hypertension, etc. (see e.g. Harrison's Principle of Internal Medicine, Eleventh Edition, McGraw-Hill Book Company, 1987 edited by E. Braunwald et al.) Furthermore, the presence of leg and ankle ulcers as well as pressure sores like decubitus may also indicate that the physical activity and/or nutritional status of an individual is sub-optimal.
- 25 In connection with treatment of wounds/ulcers debridement and wound cleansing are of particular importance. It is believed that the cleaning and/or debridement of wounds/ulcers are a prerequisite for the healing process and, furthermore, when wound healing agents are applied such agents have to exert their effect on fresh and vital tissue and not on dead tissue or

contaminated tissue. Debridement of necrotic tissue can be performed by at least four different methods: i) sharp debridement, ii) mechanical debridement, iii) enzymatic debridement, and iv) autolytic debridement.

- Sharp debridement is the most rapid method and may be the most appropriate technique for
- 5 removing areas of thick, adherent eschar and devitalized tissue in extensive ulcers. Sharp debridement is normally performed in operating room and sterile instruments are used. Furthermore, clean, dry dressing should be applied.

Mechanical debridement includes the use of wet-to-dry dressing at prescribed intervals, hydrotherapy, wound irrigation and dextranomers.

- 10 Enzymatic debridement is often used in long-term care facilities and in home care. The method is not suitable for infected ulcers which should be debrided more rapidly. Enzymatic debridement is accomplished by applying topical debriding agents to devitalized tissue on the wound's surface. Collagenase and proteases are examples of debriding agents. A clean moist dressing should be applied over the ulcer after enzyme application.
- 15 Autolytic debridement is accomplished by placing a synthetic dressing over the ulcer and allowing the eschar to self-digest through the action of enzymes normally present in the wound fluid. Autolytic debridement is contraindicated in infected ulcers.

- When the ulcer/wound has been subjected to debridement, a combination according to the invention or a composition containing the combination can be applied either directly on or into
- 20 the wound or ulcer or it can be applied in the form of a dry or moist, clean dressing into which the combination has been incorporated.

- The combination according to the invention may of course also be applied in connection with cleansing of the ulcer/wound. Ulcer wounds should be cleansed initially and at every dressing change. The process of cleansing a wound involves selecting a wound-cleansing solution and a
- 25 mechanical means of delivering that solution to the wound.

- A combination according to the present invention may be used for any wound/ulcer independent of its ethiology, nature or healing stage. It may be used for curative purposes as well as for preventive purposes. Furthermore, a combination according to the invention may be used together with other active substances such as, e.g., antiviral, antiinflammatory and/or
- 30 antibacterial substances, hormones, growth factors, enzymes, and mixtures thereof.

Examples of active substances for use together with a combination of the present invention are:

- antiviral substances: acyclovir, famciclovir, deciclovir, penciclovir, zidovudin, ganciclovir, didanosin, zalcitabin, valaciclovir, sorivudine, lobucavir, brivudine, cidofovir, n-docosanol, ISIS-2922, and prodrugs and analogues thereof, amantadin, rimantadin, foskarnet, idoxuridin,
- 5 fluoruracil, interferons and variants thereof, including alpha interferon, beta interferon, and gamma interferon, tromantadin, lentinan, levofloxacin, stavudine, tacrine, vesnarinone, ampligen, atevirdine, delavirdine, hydroxyurea, indinavir sulfate, interleukin-2 fusion toxin, seragen, lamivudine, lidakol, nevirapine, onconase, saquinavir, topotecan, verteporfin, viraplex, CMV immunoglobulin, efalith, epervudine, podophyllotoxin, proxigermanium, rifabutin,
- 10 bromovinyldeoxyuridine, ukrain, imiquimod, lamivudine, viraplex, afovirsen, amonaflide, hypericin, provir, temoporfir, aphidicolin glycinate, ibobucavir, virend, AL-721, ampligen, arildone, brivudine, CD4, 2-deoxy-D-glucose desciclovir, dichloroflavan, ditiocarb sodium, edoxudine, enviroxime, fiacitabine, inosine Pranobex, peptide T, stavudine, tribavirin, trifluridine, vidarabine, zalcitabine, and the like;
- 15 antifungal and/or antiprotozoal substances such as, e.g., miconazol, ketoconazole, clotrimazole, amphotericin B, nystatin, mepyramin, econazol, fluconazol, flucytocine, griseofulvin, bifonazole, amorolfine, mycostatin, itraconazole, terbenafine, terconazole, tolnaftate, fucidin, erythromycin, macrolides, metronidazole, and the like;
- 20 antiinflammatory substances: NSAID's such as, e.g., ibuprofen, indomethacin, naproxen, diclofenac, tolfenamic acid, piroxicam, and the like;
- antibacterial substances: antibiotic such as, e.g., amikacin, amikacin sulfate, amoxycillin, amphotomycin, ampicillin, avoparcin, azidocillin, bacampicillin, bacitracin, bakanamycin, benzanthine penicillin, benzanthine phenoxyethylpenicillin, benzylpenicillin, BL-P 1654, carbenicillin sodium, carfecillin sodium, carindacillin sodium, cefaclor, cefadroxil, cefapirin
- 25 sodium, cefatrizine, cefazaflur sodium, cefoperazone sodium, cefotaxime sodium, cefoxitin sodium, cephacetrile sodium, cephalexin, cephaloglycin, cephalonium, cephaloridine, cephalothin sodium, cephamandole, cephazolin sodium, chloramphenicol, chlortetracycline, ciclacillin, clavulanic acid, clemizole penicillin, clindamycin, clometocillin potassium, clomocycline, cloxacillin, colistin sulfate, cycloserine, demeclocycline, dicloxacillin sodium, diethanolamine
- 30 fusidate, dihydrostreptomycin sulphate, doxycycline, enramycin, epicillin, erythromycin, flucloxacillin, fosfomycin, framycetin sulphate, fusafungine, fusidic acid, gentamicin, gramicidin, hetacillin, hydramidine phenoxyethylpenicillin, josamycin, kanamycin, kitasamycin, latamoxef sodium, lincomycin, lividomycin, lymecycline, lysostaphin, meclillinam, metampicillin, methacycline, methicillin sodium, mezlocillin sodium, midecamycin, minocycline, nafcillin,

- neomycin, netilmicin sulphate, novobiocin, oleandomycin phosphate, oxacillin sodium, oxytetracycline, paromomycin sulphate, penamecillin, penethamate hydroiodide, phenethicillin potassium, phenoxymethypenicillin, piperacillin sodium, pivampicillin, pivmecillinam, polymyxin B, polymyxin B sulfate, procaine penicillin, propicillin potassium, rolitetracycline, sissomicin
- 5 sulphate, spectinomycin, spiramycin, streptomycin, sulbenicillin sodium, talampicillin, tetracycline, thiamphenicol, ticacillin sodium, tobramycin, triacetyloleandomycin, tyrothricin, vancomycin, virginiamycin and, wherever relevant, salts, complexes, prodrugs and mixtures thereof, and the like;
- hormonal substances such as, e.g., estradiol, estron, estriol, polyestradiol, polyestriol, dienestrol,
- 10 diethylstilbestrol, progesterone, dihydroergosterone, cyproterone, danazol, testosterone, and the like;
- corticosteroids such as, e.g., beclomethasone, betamethasone, betamethasone-17-valerate, betamethasone-dipropionate, clobetasol, clobetasol-17-butyrate, clobetasol-propionate, desonide, desoxymethasone, dexamethasone, diflucortolone, flumethasone, flumethasone-pivalate,
- 15 fluocinolone acetonide, fluocinonide, hydrocortisone, hydrocortisone-17-butyrate, hydrocortisone-buteprate, methylprednisolone, triamcinolone acetonide, budesonide, halcinonide, fluprednide acetate, alkometasone-dipropionate, fluocortolone, fluticasone-propionate, mometasone-furoate, desoxymethasone, diflurason-diacetate, halquinol, cliochinol, chlorchinaldol, fluocinolone-acetonid, and the like;
- 20 disinfectants like e.g. chlorhexidine, hydrogen peroxide, proflavine, cetremide, benzylperoxide, potassium permanganate, iodine, iodine derivatives, silver sulfadiazine, and hypochlorite;
- growth factors, enzymes and/or peptides such as, e.g., growth hormone releasing factors, growth factors (epidermal growth factor (EGF), nerve growth factor (NGF), TGF, PDGF, insulin growth factor (IGF), fibroblast growth factors (α FGF, β FGF, etc.), and the like), somatostatin, calcitonin,
- 25 insulin, vasopressin, interfeons, IL-2, urokinase, serratiopeptidase, streptokinase, superoxide dismutase (SOD), thyrotropin releasing hormone (TRH), luteinizing hormone releasing hormone (LH-RH), corticotrophin releasing hormone (CRF), growth hormone releasing hormone (GHRH), oxytocin, erythropoietin (EPO), colony stimulating factor (CSF), and the like.
- An important aspect of the invention is also that a composition according to the invention may
- 30 contain a free amount of first and/or second compound. Especially a free amount of a wound healing agent such as, e.g. sucrose octasulfate and/or chitosan may in certain cases be desired especially in those cases where a rapid onset of the effect is necessary or desirable or, alternatively, in those cases where a burst effect is desired.

- In those cases where the combination is applied together with at least one other active substance (as mentioned above) the dose of the other active substance is in a range necessary to obtain a therapeutic effect. Normally, the dose required is the same as in pharmaceutical compositions where no combination according to the invention is present. However, in general, the dose
- 5 necessary to obtain a suitable response (both for the combination and for any other active substance present) can be determined in an analogous manner as described below for the determination of the dose of the combination.

Promising pharmaceutical compositions according to the invention are compositions comprising a combination according to the invention together with an active drug substance which is

10 effective against any condition which may be the underlying cause of the ulcer. Such a composition would have two objectives, namely i) to impart wound healing and ii) to treat the underlying disease to the formation of ulcers. In this case, the wound is used as the application site for delivery of a further active drug substance to the circulatory system. It is contemplated that the access to the circulatory system is much easier via wounds/ulcers than via intact skin.

15 Vaccines are also interesting candidates for a delivery to the circulatory system via a wound/ulcer. Diseases like malignant melanom and psoriasis are examples of diseases which may be treated by delivery of the active drug substance via an ulcer.

- Other interesting pharmaceutical compositions are topical compositions designed for gene therapy (local or systemic).
- 20 For the administration to an individual (an animal such as a human), a combination according to the invention is preferably formulated in a pharmaceutical composition containing the composition and, optionally, one or more pharmaceutically acceptable excipients.

Thus, in one aspect the invention relates to a pharmaceutical composition comprising a combination according to the invention. In certain embodiments of the invention, a composition

25 may also contain one or more substances having any one of the activities mentioned above or any other suitable activity.

The compositions may be in form of, e.g., a spray, a solution, a dispersion, a suspension, an emulsion, tablets, capsules, pills, powders, granulates, gels including hydrogels, lotions, pastes, ointments, creams, drenches, dressings, hydrogel dressings, hydrocolloid dressings, films, foams,

30 sheets, bandages, plasters, delivery devices, suppositories, enemas, implants, aerosols, microcapsules, microspheres, nanoparticles, liposomes, and in other suitable form.

- Compositions for application to the skin or to the mucosa are considered most important in connection with the present invention. Thus, the composition comprising the combination to be administered may be adapted for administration by any suitable route, for example by topical (dermal), oral, buccal, nasal, aural, rectal, vaginal, pulmonal administration, or by
- 5 administration to a body cavity such as, e.g., a tooth root. Furthermore, a composition may be adapted to administration in connection with surgery, e.g. in connection with incision within the body in order to promote healing of internal wounds and tissue damage such as bone fractures.

The compositions may be formulated according to conventional pharmaceutical practice, see, e.g., "Remington's Pharmaceutical Sciences" and "Encyclopedia of Pharmaceutical Technology", edited
10 by Swarbrick, J. & J. C. Boylan, Marcel Dekker, Inc., New York, 1988.

As mentioned above, the application of a composition comprising a combination according to the invention is intended for skin or mucosa. Other applications may of course also be relevant such as, e.g., application on dentures, prostheses and application to body cavities such as the oral cavity. The mucosa is preferably selected from oral, nasal, aural, lung, rectal, vaginal, and
15 gastrointestinal mucosa. Furthermore, application within the dental/odontologic area is also of great importance. Relevant examples are application to periodontal (dental) pockets, to gingiva or to gingival wounds or ulcers, or in connection with dental surgery.

The pharmaceutical composition comprising a combination according to the invention serves as a drug delivery system. In the present context the term "drug delivery system" denotes a
20 pharmaceutical composition (a pharmaceutical formulation or a dosage form) which upon administration presents the active substance to the body of a human or an animal. Thus, the term "drug delivery system" embraces plain pharmaceutical compositions such as, e.g., creams, ointments, liquids, powders, tablets, etc. as well as more sophisticated formulations such as sprays, plasters, bandages, dressings, devices, etc.

25 Pharmaceutically acceptable excipients - dosage forms

Apart from the combination, the pharmaceutical compositions according to the invention may comprise pharmaceutically or cosmetically acceptable excipients. The pharmaceutically acceptable excipient for use in a composition is generally dependent on the dosage form suitable for use for a particular kind of wound/ulcer. In the following is given a detailed list of suitable
30 pharmaceutically acceptable excipients for use in compositions according to the invention.

A pharmaceutical compositions according to the invention may be adapted to administration via the oral, buccal, mucosal, nasal, rectal, vaginal, or topical route or to application directly on wounds and/or ulcers.

- In the following is given a review on relevant pharmaceutical compositions according to the
- 5 invention. The review is based on the particular route of administration. However, it is appreciated that in those cases where a pharmaceutically acceptable excipient may be employed in different dosage forms or compositions, the application of a particular pharmaceutically acceptable excipient is not limited to a particular dosage form of a particular function of the excipient.
- 10 The choice of pharmaceutically acceptable excipient(s) in a composition according to the invention and the optimum concentration thereof cannot generally be predicted and must be determined on the basis of an experimental evaluation of the final composition.

Topical compositions

- For application to the skin, the formulations according to the invention may contain
- 15 conventionally non-toxic pharmaceutically acceptable carriers and excipients including microspheres and liposomes. The compositions include creams, ointments, hydrophilic ointments, lotions, liniments, gels, hydrogels, solutions, suspensions, sticks, sprays, pastes, plasters, films, powders, soaps, shampoos, jellies, dressings such as absorbent wound dressings, pads, bandages, foams, plasters, and transdermal drug delivery systems.
- 20 The pharmaceutically acceptable excipients may include emulsifying agents, antioxidants, buffering agents, preservatives, humectants, penetration enhancers, chelating agents, gelforming agents, ointment bases, perfumes, and skin protective agents.

- Examples of emulsifying agents are naturally occurring gums, e.g. gum acacia or gum tragacanth; naturally occurring phosphatides, e.g. soybean lecithin; sorbitan monooleate
- 25 derivatives; wool fats; wool alcohols; sorbitan esters; monoglycerides; and fatty alcohols.

Examples of antioxidants are butylated hydroxy anisole (BHA), ascorbic acid and derivatives thereof, tocopherol and derivatives thereof, butylated hydroxy anisole, and cysteine.

- Suitable examples of preservatives for use in compositions according to the invention are parabens, such as methyl, ethyl, propyl p-hydroxybenzoate, butylparaben, isobutylparaben,
- 30 isopropylparaben, potassium sorbate, sorbic acid, benzoic acid, methyl benzoate, phenoxyethanol, bronopol, bronidox, MDM hydantoin, iodopropynyl butylcarbamate, EDTA, propylene glycol

(increases the solubility of preservatives) benzalconium chloride, and benzylalcohol, or mixtures of preservatives like Germaben II & IIE (mixture of imidazolidinyl urea, methyl and propyl parabens and propylene glycol; available from ISP-Sutton Labs.)

Examples of humectants are glycerin, propylene glycol, sorbitol, lactic acid, and urea.

- 5 Examples of chelating agents are sodium EDTA, citric acid, and phosphoric acid.

Examples of other excipients are edible oils like almond oil, castor oil, cacao butter, coconut oil, corn oil, cottonseed oil, linseed oil, olive oil, palm oil, peanut oil, poppyseed oil, rapeseed oil, sesame oil, soybean oil, sunflower oil, and teaseed oil; and of polymers such as carmelose, sodium carmelose, hydroxypropylmethylcellulose, hydroxyethylcellylose, hydroxypropylcellulose,

- 10 pectin, xanthan gum, carrageenan, locust bean gum, acacia gum, gelatin, carbomer, emulsifiers like vitamin E, TPGS, glyceryl stearates, cetanyl glucoside, and alginates.

Examples of ointment bases, in general, are beeswax, paraffin, cetanol, cetyl palmitate, vegetable oils, sorbitan esters of fatty acids (Span), polyethylene glycols, and condensation products between sorbitan esters of fatty acids and ethylene oxide, e.g. polyoxyethylene sorbitan

- 15 monooleate (Tween).

Examples of hydrophobic or water-emulsifying ointment bases are paraffins, vegetable oils, animal fats, synthetic glycerides, waxes, lanolin, and liquid polyalkylsiloxanes.

Examples of hydrophilic ointment bases are solid macrogols (polyethylene glycols).

- Other examples of ointment bases are triethanolamine soaps, sulphated fatty alcohol and
20 polysorbates.

Examples of gel bases or components which are able to take up exudate from a wound/ulcer are: liquid paraffin, polyethylene, fatty oils, colloidal silica or aluminium, zinc soaps, glycerol, propylene glycol, tragacanth, starch, cellulose derivatives, carboxyvinyl polymers, magnesium-aluminium silicates, Carbopol, hydrophilic polymers such as, e.g. starch or cellulose derivatives,

- 25 liquid absorbing wound bandages, water-swellable hydrocolloid, and alginates.

Examples of powder components are: alginate, collagen, lactose, powder which is able to form a gel when applied to a wound/ulcer (absorbs liquid/wound exudate). Normally, powders intended for application on large open wounds must be sterile and the particles present must be micronized.

Alginic acid or alginates are important excipients in connection with pharmaceutical compositions according to the present invention. As explained above, alginates can form a gel and, furthermore, they can absorb wound exudates and thus contributing to controlling the moisture content of a wound. Alginic acid and alginates are available in various qualities having

5 a mean molecular weight in the range of e.g. about 32,000-200,000. The relative proportion of mannuronic and guluronic acid residues varies from one quality to another.

Other excipients for use in topical compositions include tackifier resin, viscous elastomeric binders, elastic film, elastic adhesive material, elastomers and plasticizers.

Dressings and/or bandages are also important delivery systems for a combination according to

10 the invention. Dressings may be in the form of absorbent wound dressings for application to exuding wounds. Such dressings are frequently made of cotton or viscose fibres which are enclosed in a sleeve of gauze or a suitable non-woven fabric. Other relevant materials are cellulose fibres, cellulose wood pulp (fine powdery material). Dressings may also be in the form of hydrogel dressings such as, e.g., i) dressings having a fixed three-dimensional macro-

15 structure, and ii) dressings involving amorphous hydrogels. In the latter case, the dressings progressively decrease in viscosity when the material absorbs fluid, and the dressing may then flow on top of the wound and take on the shape of the wound. Examples of other kinds of dressings are i) hydrocolloid dressings (gel-forming agents combined with other material such as, e.g. elastomers and adhesives) including hydrocolloid granules or paste and hydrocolloid sheet,

20 ii) alginate sheet (rope or ribbon, alginate with integral absorbent pad), iii) foams (foam dressings, silastic foam, polyurethane foam), iv) various polysaccharide materials, v) occlusive dressings, vi) semipermeable dressings, vii) paraffin gauze dressings, viii) Tulle dressings, ix) polysaccharide pastes, granules and beads (may be manufactured from dextran derivatives), and x) odour-absorbing dressings. Suitable bandages may be i) non-extensible bandages, ii)

25 extensible bandages, iii) adhesive/cohesive bandages, iv) tubular bandages, v) medicated paste bandages, and vi) orthopaedic casting materials.

Semipermeable films and thin foam sheets have little or no intrinsic absorbent capacity. They are permeable to moisture vapour when placed on a wound and, accordingly, the aqueous component of a wound exudate is lost through the backing of the dressing in the form of a

30 vapour. The cellular material remains trapped at the surface of the wound.

Alginate and hydrocolloid dressings take up wound exudate when placed on a wound. When doing so they produce an aqueous gel on the surface of the wound and this gel is believed to be beneficial for the healing of the wound due to the retaining of moisture in the wound.

- The compositions mentioned above for topical administration are most suitably for application directly to wounds or they may be suitable for application to or for introduction into relevant orifice(s) of the body, e.g. the rectal, urethral, vaginal, aural, nasal or oral orifices. The composition may simply be applied directly on the part to be treated such as, e.g., on the
- 5 mucosa, or by any convenient route of administration.

Fluid/liquid compositions for oral use or for application to mucosa or skin

- Compositions like a suspension, an emulsion or a dispersion provide the combination in admixture with a dispersing or wetting agent, suspending agent, and/or one or more preservatives. Such compositions may also be suitable for use in the delivery of the combination to
- 10 e.g. an intact or damaged mucosa such as the gastrointestinal, buccal, nasal, rectal, or vaginal mucosa, or for administration to intact or damaged skin, or wounds/ulcers.

- Suitable dispersing or wetting agents are, for example, naturally occurring phosphatides, e.g., lecithin, or soybean lecithin; condensation products of ethylene oxide with e.g. a fatty acid, a long chain aliphatic alcohol, or a partial ester derived from fatty acids and a hexitol or a hexitol
- 15 anhydride, for example polyoxyethylene stearate, polyoxyethylene sorbitol monooleate, polyoxyethylene sorbitan monooleate, etc.

- Suitable suspending agents are, e.g., naturally occurring gums such as, e.g., gum acacia, xanthan gum, or gum tragacanth; celluloses such as, e.g., sodium carboxymethylcellulose, microcrystalline cellulose (e.g. Avicel® RC 591, methylcellulose; alginates such as, e.g., sodium alginate, etc.
- 20 Suitable examples of preservatives for use in compositions according to the invention are the same as those mentioned above.

Rectal and/or vaginal compositions

- For application to the rectal or vaginal mucosa, suitable compositions according to the invention include suppositories (emulsion or suspension type), enemas, and rectal gelatin capsules
- 25 (solutions or suspensions). Appropriate pharmaceutically acceptable suppository bases include cocoa butter, esterified fatty acids, glycerinated gelatin, and various water-soluble or dispersible bases like polyethylene glycols and polyoxyethylene sorbitan fatty acid esters. Various additives like, e.g., enhancers or surfactants may be incorporated.

Nasal compositions

- For application to the nasal mucosa, nasal sprays and aerosols for inhalation are suitable compositions according to the invention. In a typical nasal composition, the combination is present in the form of a particulate formulation optionally dispersed in a suitable vehicle. The pharmaceutically acceptable vehicles and excipients and optionally other pharmaceutically acceptable materials present in the composition such as diluents, enhancers, flavouring agents, preservatives, etc. are all selected in accordance with conventional pharmaceutical practice in a manner understood by the persons skilled in the art of formulating pharmaceuticals.
- 5

After administration of a nasal composition according to the invention, the active substance may be adsorbed on the nasal mucosa. The adsorption on the mucosa is believed to lead to a less irritative effect than when, e.g., a liquid vehicle, e.g. containing a penetration enhancer or promoter, is employed.

Compositions for oral use

Compositions according to the invention may also be administered by the oral route. Suitable oral compositions may be in the form of a particulate formulation or in the form of a solid, semi-solid or liquid dosage form.

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Compositions for oral use include solid dosage forms such as, e.g., powders, granules, sachets, tablets, capsules, effervescent tablets, chewable tablets, lozenges, immediate release tablets, and modified release tablets as well as fluid or liquid formulations such as, e.g. powders, dispersible powders, or granules suitable for preparation of an aqueous suspension by addition of an aqueous medium, emulsions, dispersions, and mixtures.

Solid dosage forms for oral use

The composition contain the combination and any further active substance optionally in admixture with one or more pharmaceutically acceptable excipient. These excipients may be, for example,

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inert diluents or fillers, such as sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate;

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granulating and disintegrating agents, for example, cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid;

binding agents, for example, sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone such as, e.g., PVP K12, PVP K15, PVP K17, PVP K25, PVP K30, PVP K60,

- 5 PVP K90, or PVP K120, or combinations thereof, polyvinylacetate, or polyethylene glycol; and

lubricating agents including glidants and antiadhesives, for example, magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc.

Other pharmaceutically acceptable excipients can be colorants, flavouring agents, plasticizers, humectants, buffering agents, etc.

- 10 In those cases where the pharmaceutical composition is in the form of a solid dosage form in unit dosage form (e.g. a tablet or a capsule), the unit dosage form may be provided with a coating like one or more of the coatings mentioned below.

In those cases where the composition is in the form of a tablet, capsule or a multiple unit composition, the composition or the individual units or a tablet or a capsule containing the

- 15 individual units may be coated e.g. with a sugar coating, a film coating (e.g. based on hydroxypropyl methylcellulose, methylcellulose, methyl hydroxyethylcellulose, hydroxypropylcellulose, carboxymethylcellulose, acrylate copolymers (Eudragit), polyethylene glycols and/or polyvinylpyrrolidone) or an enteric coating (e.g. based on methacrylic acid copolymer (Eudragit), cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, 20 hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, shellac and/or ethylcellulose). Furthermore, a time delay material such as, e.g., glycetyl monostearate or glycetyl distearate may be employed.

In a pharmaceutical composition according to the invention, a combination is generally present in a concentration in a range of from about 0.01% to about 99.9% w/w. The amount of

- 25 composition applied will normally result in an amount of the second compound per cm² wound/skin/tissue area corresponding to from about 0.1 mg/cm² to about 500 mg/cm² such as from about 1 mg/cm² to about 100 mg/cm². In those cases where the second compound is sucrose octasulfate the amount of composition applied will normally equals an amount of sucrose octasulfate per cm² wound/skin/tissue area corresponding to from about 0.1 mg/cm² to about 30 100 mg/cm², such as from about 0.1 mg/cm² to about 10 mg/cm². The amount applied of the composition depends on the load and the release rate of e.g. the sucrose octasulfate on the first compound (e.g. chitosan), but is generally in a range corresponding to at the most 5 mg/cm².

The concentration of the combination in a pharmaceutical composition depends on the nature of the second compound in question, its potency, the severity of the disease to be prevented or treated, and the age and condition of the patient. Methods applicable to selecting relevant concentrations of the combination in the pharmaceutical composition are well known to a person skilled in the art and may be performed according to established guidelines for good clinical practice (GCP) or Investigational New Drug Exemption ("IND") regulations as described in e.g. Drug Applications, Nordic Guidelines, NLN Publication No. 12, Nordic Council on Medicines, Uppsala 1983 and Clinical Trials of Drugs, Nordic Guidelines, NLN Publication No. 11, Nordic Council on Medicines, Uppsala 1983. A person skilled in the art would, by use of the methods described in standard textbooks, guidelines and regulations as described above as well as common general knowledge within the field, be able to select the exact dosage regimen to be implemented for any combination and/or selected active substance and dosage form using merely routine experimentation procedures.

In another aspect, the invention relates to a method for the preparation of a combination, the method comprising mixing the first and the second compound in a suitable medium such as an aqueous medium and, optionally, adjusting the pH in order to precipitate the combination. The precipitate is collected and, optionally, purified. Other methods, e.g., involving the preparation of a film, are apparent from the experimental section. It is contemplated that the loading of the second compound in the resulting combination as well as the dominating structure of the combination is dependent on the conditions prevailing during the reaction. Furthermore and as will appear from the experimental section herein, the reaction conditions are also believed to influence the composition and the yield of the combination obtained such as, e.g., the method of preparation (e.g. the mixing process, the mixing process and the order in which the components is added, the ionic strength prevailing in the reaction mixture, the viscosity, the kind of solvent chosen for the reactants, any addition of accelerators, the ratio of SOS and chitosan, the pH prevailing in the reaction mixture, reaction time, rotation speed, e.g. from 0-1000 rpm, homogenisation of the reaction mixture, heating/cooling of the reaction mixture, employment of ultrasonic treatment (cf. Example 5), the starting materials (chitosan, chitosan glutamate, chitosan chloride, chitosan lactate, sucrose octasulfate as hydrate or other solvates or as a sodium, potassium or another salt, or derivatives thereof) and the concentration thereof, the precipitation method, any purification step, etc.)

As will be understood, details and particulars concerning the method aspect of the invention will be the same as or analogous to the details and particulars concerning the combination and composition aspects discussed above, and this means that wherever appropriate, the statements above concerning the combination or composition, their preparation, improved properties and

uses apply mutatis mutandis to the methods for the preparation as well as the use aspects of the invention.

In another aspect of the invention the second compound is an active drug substance. Thus, the invention relates also to a combination of a first compound of an oligo- or polysaccharide containing aminosugar units and a second compound of an active drug substance. Such a combination is also not merely a physical blend but a reaction has taken place between the first and the second compound. Thus, in those cases where the second compound is an active drug substance, the combination may as well be a salt, a complex such as an inclusion complex, or the compounds may be held together in a polymeric network or as a mechanical inclusion, or the compounds may even be covalently bound together in the combination, or a combination of the above-mentioned mechanisms may be involved.

A combination as defined above must fulfil the same requirements as described above under the aspect of the invention relating to combinations wherein the first compound is as defined above but the second compound is a sulfated mono-, di- or oligosaccharide.

It is believed that when the second compound of a combination according to the invention is an active drug substance administration of the combination to a mammal, in particular a human, will result in a controlled release of the active drug substance due to a controlled release of the active substance from the combination (as discussed herein). Furthermore, the combination may be bioadhesive due to the properties of the first compound (which e.g. may be a chitosan as described above). Administration of a bioadhesive combination may enable a localized effect as the combination is maintained at the application site by means of bioadhesion and from that site releases the active drug substance in a controlled manner.

It is contemplated that the following active drug substances are able to form a combination as defined above when reacted with a first compound as defined above. A characteristic of the active drug substances relevant in the present context as second compounds is that the active drug substances contain a carboxylic acid or a sulfonic acid group.

Examples of active drug substances which are useful as second compounds in a combination according to the invention:

Anti-inflammatory agents/analgesics:

- 30 Salicylic acid
- 5-aminosalicylic acid
- Acetylsalicylic acid (aspirin)

- Paracetamol
- Aceclofenac
- Acemetacin
- Acetylsalicylic acid
- 5 5-Adenosylmethionine
- Alminoprofen
- Benoxyprofen
- Bermoprofen
- Bromfenac
- 10 Bucloxic acid
- Bumadizum
- Butibufen
- Carprofen
- Cinmetacin
- 15 Clometacin
- Clomixin
- Clopirac
- Ibuprofen
- Ibufenac
- 20 Indomethacin
- Indoprofen
- Isofezolac
- Isoxepac
- Diclofenac
- 25 Diflunisal
- Dipyrrocetyl
- Enfenamic acid
- Enoxolone
- Etodolac
- 30 Fenbufen
- Fencloxic acid
- Fentiazac
- Flufenamic acid
- Flunixil
- 35 Flunoxaprofen
- Flurbiprofen
- Fosfosal
- Gentisic acid

- Ketorolac
- Ketoprofen
- Lonazolac
- Loxoprofen
- 5 Mefenamic acid
- Nadifloxacin
- Nalidixic acid
- Naproxen
- Oxaprozin
- 10 Pirazolac
- Pirprofen
- Pranoprofen
- Protizinic acid
- Salicylamide O-acetic acid
- 15 Salicylsulfuric acid
- Salsalate
- Suprofen
- Tiaprofenic acid
- Tolmetin
- 20 Tropesin
- Ximoprofen
- Zaltoprofen
- Zomepirac
- Morphine (bearing a phenol group), and
- 25 active drug substances classified as Non-Steroidal Anti-inflammatory Drugs (NSAIDs)

- Penicillins:
- Amdinocillin
 - Amdinocillin Pivoxil
 - Aspoxicillin
 - 30 Azidocillin
 - Benzylpenicillin sodium
 - Benzylpenicillin acid
 - Carindacillin
 - Carpetimycins
 - 35 Carbenicillin
 - Benzylpenicillin
 - Benzylpenicillinprocain

- Phenoxymethypenicillin
Dicloxacillin
Cloxacillin
Flucloxacillin
5 Meticillin
Amoxicillin
Ampicillin
Bacampicillin
Pivampicillin
10 Piperacillin
Mecillinam
Hетациллин
Oxacillin
Panipenem
15 Penicillin G Benethamine
Penicillin G Benzathine
Penicillin N
Penicillin O
Piperacillin
20 Pivcefalexin
Propicillin
Quinacillin
Ritipenem

Antibiotics:

- 25 Acediasulfone
Alclofenac
Aztreonam
Benzyl isothiocyanate
Benzoylpas
30 Carumonam
Cefaclor
Cefadroxil
Cefamandole
Cefatrizine
35 Cefazedone
Cefazolin
Cefbuperazone

- Cefcapene pivoxil
- Cefclidin
- Cephalexin
- Cinoxacin
- 5 Ciprofloxacin
- Enoxacin
- Enrofloxacin
- Flomoxef
- Flumequine
- 10 Difloxacin
- Fusidic acid
- Grepafloxacin
- Lornafloxacin
- Lymecycline
- 15 Merbromin
- Mupirocin
- Nifuroquine
- Ofloxacin
- Opiniazide
- 20 Pefloxacin
- Phthalylsulfathiazole
- Pipemidic acid
- Pyritohione (also active as an antifungal agent)
- Rosoxacin
- 25 Rufloxacin
- Salazosulfadimidine
- Succinylsulfathiazole
- Succisulfone
- Tigemonam
- 30 Trovalfloxacin

Antihypertensive agents:

- Alacapril
- Candesartan
- Carmoxirole
- 35 Cilazapril
- Delapril
- Eprosartan

Fosinopril

Lisinopril

Moveltipril

Perindopril

5 Ramipril

Valsartan

Antirheumatics:

Allocupreide sodium

Bucillamine

10 Clobuzarit

Lobenzanit

Thyroid inhibitors:

3,5-diiodotyrosine

Tiratricol (antihypothyroid)

15 Antiparkinsonian:

Droxidopa

Anesthetics (topical):

Egonine

Antineoplastic agents:

20 Chlorambucil

Denopterin

Eflomithine

Lonidamine

Mycophenolic acid

25 Podophyllic acid

Streptonigrin

Ubenimex

5-fluoruracil (NH-acidic compound)

Agents for treatment of diabetic neuropathy:

30 Epalrestat

Uricosuric agents:

Ethebenicid

Diuretics:

Acefulline

Bumetanide

5 Mercumallylic acid

Mersalyl

Piretanide

Probenecid

Ticrynafen

10 Choleretic agents:

Cholic acid

Clanobutin

Cyclobutyrol

Dehydrocholic acid

15 Fencibutirol

Florantyrone

Antihistamines:

Bietanantine

Cetirizine

20 Fexofenadine

Acrivastine

Tranilast

Antispasmodic/choleretic agents:

Trepibutone

25 Antithrombotic agents:

Daltroban

Indobufen

Isbogrel

Ozagrel

30 Tirofiban

Triflusal

Antiasthmatic agents:**Montelukast****Seratrodast****Agents for treatment of infertility in mares:**

- 5 **Fluprostenol**

Vitamins:**Folic acid****Pantothenic acid****Vitamin A acid**

- 10 **Antidote to folic acid antagonist, antianemic (folate deficiency):**

Folinic acid**Antiarthritic agents:****Diacerein****Anticonvulsant agents:**

- 15 **Gabapentin**

Sclerosing agents:**2-hexyldecanoic acid****CNS stimulants:****Amineptine**

- 20 **Hexacyclonate sodium**

Anthelmintics:**Kainic acid****Netobimin****Stibocaptate**

- 25 **Antiangular:**

Limaprost**ACE inhibitors:****Captopril**

- Benazepril
- Enalapril
- Perindopril
- Trandolapril
- 5 Moexipril
- Fosinopril
- Ramipril
- Lisinopril

Antihyperlipoproteinemic agents:

- 10 Acifran
- Acipimox
- Ciprofibrate
- Clinofibrate
- Clofibrlic acid
- 15 Pravastatin sodium
- Fluvastatin
- Gemfibrozil
- Meglutol
- Nicotinic acid
- 20 Oxiniacic acid

Antiamoebic agents:

Thiocarbamizine

Luteolytic agents:

Tiaprost

25 Antiuclcerative agents:

- Arbaprostil
- Carbenoxolone
- Cetraxate
- Rebamipide
- 30 Rosaproston
- Rotraxate
- Sofalcone
- Trimoprostil

Antiviral agents:

Statine

Antidepressants:

Tianeptine

5 Anticonvulsants:

Tiagabine

Mucolytic agents:

Stepronin

Antidiabetic agents:

10 Repaglinide

Tolrestat

Zopolrestat

Agents for ulcerative colitis/Chrons' disease:

Balsalazine

15 IpsiSalazine

Sulphasalazine

Sulfasalazine

Mesalamine

Olsalazine

20 5-aminosalicylic acid

Antiepilectics:

Phenytoin

Agents for asthma bronchialis and other broncho-spasms:

Theophyllin

25 Immunomodulators:

Pidotimod

Procodazole

Antifungals:

Ujothion

Antiglaucoms:**Unoprostone****Others:****Thromboxanes**5 **Prostaglandin E2****Prostaglandin E1, F2alpha****L-Dopa****Hydantoins****Allopurinol**10 **Diacerein****Acetretin****Aclatonium napadisilate****Actarit****Artesunate**15 **Benfurodil hemisuccinate****Benztropine mesylate****Calcium N-carbamoylaspartat****Calcium 2-ethylbutanoate****Capobenic acid**20 **Carboprost****Chenodiol****Clorazepic acid****Cromolyn****Especially interesting combinations according to the invention are combinations wherein the**25 **first compound is chitosan and the second compound is any of the above-mentioned active drug substances or combinations thereof.****In general, combinations according to the invention wherein the second compound is an active drug substance as defined above are contemplated to act as prodrugs of the parent active drug substance and/or a synergistic effect between the first and the second compound may be**30 **achieved. This means that combinations wherein the second compound is an active drug substance as defined above are suitable for the same therapeutic or prophylactic purpose as the parent drug substance. The active drug substance may also be effective while being maintained in the combination; thus, e.g. fragments of the active drug substance may extend out from the combination and lead to a therapeutic effect. Accordingly, this aspect of the invention is not**

restricted to combinations which are suitable for use as wound healing agents but for any appropriate therapeutic/prophylactic use.

In those cases in which the combination according to the invention acts as a prodrug of the parent active drug substance, a person skilled in the art will have no difficulties in determining
5 the dose of the combination to be administered to a mammal, in particular to a human, and in what form the dosage should be presented (e.g. compositions suitable for oral, nasal, topical, parenteral etc. administration).

In a still further aspect the invention also relates to a method for the preparation of the above-mentioned combinations wherein the second compound is an active drug substance as defined
10 above and to pharmaceutical compositions containing the combinations together with at least one acceptable pharmaceutical excipient.

As will be understood, details and particulars concerning the first aspect of the invention (i.e. a combination between a first and a second compound, wherein the first compound is an oligo- or polysaccharide containing aminosugar units and the second compound is a sulfated mono-, di- or
15 oligosaccharide) will be the same or analogous to the details and particulars concerning the other aspects discussed above, and this means that whenever appropriate, the statements above concerning combinations of an oligo- or polysaccharide containing aminosugar units and a sulfated mono-, di- or oligosaccharide, their preparation, improved properties and uses apply
mutatis mutandis to the combinations wherein the second compound is an active drug substance
20 as defined above, to pharmaceutical compositions containing such combinations and to methods for the preparation of such combinations.

The invention is further illustrated by the working examples described in the following.

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 shows a thermogram of sodium sucrose octasulfate. The thermogram was recorded on a
25 Perkin Elmer Analysis 7 Series System using 6.58 mg of sodium sucrose octasulfate. The start temperature was 20°C and the end temperature 200°C. The plot shows the heat flow in mW against the temperature (°C).

Fig. 2 shows a thermogram of chitosan. The experimental details are as described under Fig. 1. 1.600 mg of chitosan was used.

Fig. 3 shows a thermogram of a combination of chitosan and SOS (batch No. AAAS-2-1). The experimental details are as described under Fig. 1. 5.640 mg of chitosan-SOS was used.

Fig. 4 shows a thermogram of a physical blend of chitosan and sodium sucrose octasulfate. The experimental details are as described under Fig. 1. The physical blend used consisted of about 4
5 mg chitosan and about 21 mg sodium sucrose octasulfate.

Fig. 5 shows the results of a number of syntheses of chitosan-SOS in which the initial concentration of sodium sucrose octasulfate has been varied in order to determine whether different loads of SOS are obtained. The graph is a plot of the loading of sodium sucrose octasulfate (Na-SOS) to chitosan (solution of Protasan CL 210 2 mg/ml) versus the initial
10 concentration of sodium sucrose octasulfate in the SOS solution used in the reaction mixture.

MATERIAL AND METHODS

Materials

The following qualities of a poly-D-glucosamine, i.e. chitosan, have been used in the Examples described below:

- 15 * Chitosan base available from Sigma, practical grade
* Seacure CL 310, Pronova Biopolymer a.s., Norway, (viscosity 445 mPas, a deacetylation grade about 82 % and a mean molecular weight of about 10-1000 kDalton)
* Seacure CL 313, Pronova Biopolymer a.s., Norway, (a deacetylation grade of about 84%)
* Seacure 110 G, Pronova Biopolymer a.s., Norway, (a deacetylation grade about 75-85%
20 and a mean molecular weight of about 50-200 kDalton)
* Protasan CL 210, Pronova Biopolymer a.s., Norway, (viscosity 98 mPas and a deacetylation grade of about 84%)
* Protasan G210, Pronova Biopolymer a.s., Norway, (viscosity 125 mPas, a deacetylation grade of about 80%)
25 * Protasan G110, Pronova Biopolymer a.s., Norway, (viscosity 10mPas)

The different grades of chitosan and chitosan salts are specified by the mean molecular weight, the degree of deacetylation and viscosity of the solutions. The viscosities given above are data from the supplier (1% solution in 1% w/v acetic acid, Brookfield LVT viscometer, 25°C, 30 rpm)). Pronova Biopolymer a.s., Norway supplies two chitosan salts, namely the glutamate and
30 the hydrochloride salt, both in two different qualities. Protasan is a very purified product and

Seacure is a product of regular quality. The degree of deacetylation of chitosan and chitosan salts is normally about 84%

- * Sodium sucroseoctasulfate available from BM Research, Denmark, water content of about 15% w/w (purity is at least 95 % calculated on the dried substance)
- 5 * Acetic acid available from Bie & Berntsen, Denmark
- * Hydrochloric acid available from Bie & Berntsen, Denmark
- * Sodium chloride available from Bie & Berntsen, Denmark
- * Potassium chloride available from Bie & Berntsen, Denmark
- * Sodium hydroxide available from Bie & Berntsen, Denmark
- 10 * Dimethylsulfoxide available from Struers, Denmark
- * Ammoniumsulfate available from Bie & Berntsen, Denmark
- * Lysozyme L 6876 available from Sigma (activity: 50,000 units per mg protein)
- * Carbopol available from BF Goodrich, U.S.A.
- * Polysorbate 80 (Tween® 80) from Kemi-Intressen
- 15 * Cetylano (cetylano) from Unikem A/S, Denmark
- * Paraffinum liquidum from Unikem A/S, Denmark
- * Glycerolmonostearate 40-50 from Unikem A/S, Denmark
- * Methylparahydroxybenzoate from Unikem A/S, Denmark
- * Glycerol from Joli Handel AP, Denmark
- 20 * Sorbitol from Merck, Denmark
- * Methyl cellulose (Methocel MC) from Fluka (available form Bie & Berntsen, Denmark)
- * Protosan available from Pronova Biopolymer A.S., Norway
- * Polyethylene from Merck, Denmark
- * Lactose from Unikem A/S, Denmark
- 25 * Cellulose from Fluka (available from Bie & Berntsen, Denmark)
- * Carboxymethylcellulose sodium salt from Fluka (available from Bie & Berntsen, Denmark)
- * Hydroxypropylcellulose from Aldrich (available from Wolff & Kaaber Trading ApS, Denmark)
- 30 * Tragacanth from Sigma (available from Bie & Berntsen, Denmark)
- * Gelatine (Gelatinum cr. pulv.) from Deutsche Gelat.
- * Chondrus (Chondroitin sulfate or chondrosine) from Merck (available from Bie & Berntsen, Denmark)
- * Sodium alginate from Sigma
- 35 * Collagen from Merck
- * Glucose from Cerestar Scandinavia A/S
- * Potassium sorbate from Nordisk Droege Handel A/S, Denmark

- * EDTA disodium from Unikem A/S
- * Isopropyl alcohol from Merck
- * Phosphoric acid from Merck
- * Arlacel 1689 from ICI (available through Kemi-Interessen, Denmark)
- 5 * Arlamol E from ICI
- * Magnesium stearate from AKCROS BV
- * Tocopheryl acetate from Merck
- * Pantheol from Fluka

All substances were of pharmacopoeial grade, if possible.

10 Apparatus

Differential Scanning Calorimeter, DSC-7 from Perkin Elmer

HPLC:

Pump LC-6A from Shimadzu

Autosampler SCL-6B from Shimadzu

15 Refractive index detector RID-6a from Shimadzu

Mobilephase, 0.595 M $(\text{NH}_4)_2\text{SO}_4$, pH 3.5

Shaking table from Edmund Bühler

Coulter Multisizer II (Coulter), Malvern 2600 droplet and particle size analyse (for the determination of particle size distribution).

20 Strölein Areameter and Coulter S3100 for the determination of the surface area of the particles.

Methods

Determination of viscosity

- The dynamic viscosity of a test sample or a composition (e.g. solutions, ointments, gels, creams, oils, lotions, foams, etc. with or without a content of a combination of chitosan-SOS according to 25 the invention) is determined using a RheoStress RS 100 Rheometer, HAAKE (Germany) equipped with a RS 100 1.2 software package. The measurement can be performed at 20°C** \pm 0.5°C or, alternatively, at 25°C or at 37°C \pm 0.5°C or at varying temperatures in order to obtain temperature curves. The following conditions can be used: probe: i) plate and plate (d=20mm)

shear rate 1-300 sec⁻¹ (flow curve) gab: 0.3-1 mm, ii) plate and cone (d=60 mm) shear rate 1-300 sec⁻¹. The viscosities can be read at different times, e.g. at t=180 sec.

EXAMPLES

EXAMPLE 1

5 Preparation of chitosan solutions

Chitosan base is not soluble at pH above 7.0 and, therefore, the pH must be maintained at pH 6.0 or below to avoid precipitation.

- A 1% w/v solution is prepared by slurring e.g. 2 g chitosan (Sigma, practical grade) in 100 ml of distilled water followed by adding 100 ml of a 2% w/v solution of acetic acid. The resulting mixture, i.e. a 1% w/v solution of chitosan in 1% w/v acetic acid, is stirred vigorously for 60 minutes or until dissolution has taken place. Heating the solution can accelerate the dissolution process, but prolonged heating may result in a decrease in viscosity. Alternatively, chitosan can be dissolved directly by adding chitosan to a 1% w/v acetic acid solution. 2% w/v solutions of chitosan (Sigma, practical grade) in 1% w/v can also be prepared.
- 15 Alternatively, chitosan may be dissolved under vigorous stirring for 24-48 hours.

1% w/v and 2% w/v solutions of chitosan glutamate and chitosan hydrochloride can easily be made in water.

EXAMPLE 2

Formation of chitosan-SOS beads

- 20 A solution of 1% w/v (10 mg/ml) chitosan (Sigma) in 1% w/v acetic acid is pumped dropwise through a tip from a microliter pipette (Finntip 60) to a solution of 12.5 mg/ml sucrose octasulfate sodium salt (SOS-Na₈) in distilled water.

Beads (drops or pearls) appear in the solution as chitosan gets into contact with SOS-Na₈ presumably forming a combination or complex on the surface of the chitosan beads. The beads 25 are approximately 2 mm in diameter and the surface becomes opaque whitish (dependent on the concentration of SOS-Na₈ in the solution). Upon resting in the solution the beads lose their surface tension and shrink. The beads are then filtered and washed with distilled water followed

by drying at 40°C in an oven. The dry product appears as shrivelled beads. It is a very hard material which is not easily pulverized.

EXAMPLE 3

Preparation of a combination of chitosan and SOS-Na₈

- 5 An aqueous solution containing 12.5 mg sucrose octasulfate sodium salt (SOS-Na₈) is added to a solution of 1% w/v chitosan in 1% w/v acetic acid under vigorous stirring. A flocculate or flake-like precipitate is obtained. The dried product appears as a very stiff material which is difficult to pulverize.

EXAMPLE 4

10 Preparation of a combination according to the invention

In the following general methods for the preparation of combinations according to the invention are described.

In the following details are given on the preparation of various chitosan-sodium sucroseoctasulfate combinations.

15 Solutions used:

- I Solutions of chitosan (deacetylation grade 75-85%, concentration: 1% w/v (10 mg/ml) in 1% w/v acetic acid; the chitosan employed may be either chitosan base, chitosan hydrochloride or chitosan glutamate. In the following chitosan has been employed as the base unless otherwise specified.
- 20 II Aqueous solutions of sodium sucrose octasulfate in a concentration range of from about 1.25 mg/ml to about 12.5 mg/ml (corresponding to from about 0.875 mg/ml SOS⁻⁸ ions to about 8.75 mg/ml SOS⁻⁸ ions).

Preparation conditions (3 methods used):

- Method I 10 ml of I (se above) is added slowly and dropwise to 40 ml of II at room temperature. The 10 ml of I is added over a time period of 40 min. The reaction time is 40 min. Small transparent spheres containing the chitosan solution are formed. The chitosan solution reacts slowly with sucrose octasulfate on the surface
- 25

of the drops and small spheres are formed and filtered off. The thus collected spheres are dried at room temperature for 24 hours and dried flakes are obtained.

Method II 40 ml of II (5 mg/ml) is added to 10 ml of I in a continuous thin jet through a tip for a microliter pipette (Finnpipette 60) with continuously stirring with a Heidolph R2R-2000 stirrer (200-750 rpm). The formed combination (concentrated around the rotor-blades) is isolated and dried at room temperature for 24 hours.

Method III 10 ml of I is placed in a petri dish. The solution is dried 4 hrs in a fume cupboard or for 2 hours at 60°C. The chitosan solution is dried in an oven (40-60°C) until nearly dryness and a soft film is obtained. Alternatively, the chitosan solution is dried to dryness either at room temperature or in an oven (conditions as above) and if a crisp film is obtained, the film can easily be softened with water. To the thus obtained chitosan film, 20 ml of II (12.5 mg/ml) is added and left to evaporate the liquid,

A lot of batches were made after these 3 methods. The use of the same method given above for the preparation of a combination of chitosan and SOS resulted in products having the same visual appearance, but the appearance of the products differed dependent of the individual method applied. The batches were investigated on Differential Scanning Calorimetry DSC (see below) and all batches show thermal properties that are different from those of the pure materials and a physical blend of chitosan and SOS-Na₈. In the following table is given a review of the method used for the preparation of various batches of chitosan-SOS combinations:

Method I	Method II	Method III
AAAS5-2	AAAS5-1	AAAS13b-4
AAAS7-2	AAAS11-3	AAAS13c-3
AAAS7-6	AAAS11-6	AAAS15-1
25 AAAS12-4		AAAS15-3
AAAS12-6		

AAAS5-1: 0.5 g SOS-Na₈ dissolved in 100 ml of distilled water is added to 50 ml of a chitosan solution (cf. Example 1) by means of a syringe and a canula and with vigorously stirring. The precipitate is filtered off and dried at ambient temperature.

30 AAAS5-2: As AAAS5-1 but the chitosan solution is added to the SOS-Na₈-solution.

AAAS7-2: 10 ml of a chitosan solution (cf. Example 1) is dropwise added to 100 mg SOS-Na₈ in 40 ml distilled water.

AAAS7-6: 10 ml of a chitosan solution (cf. Example 1) is dropwise added to 0.5 g SOS-Na₈ in 40 ml distilled water.

- 5 AAAS11-3: 0.100 g SOS-Na₈ in 40 ml distilled water is slowly added through a tip for a pipette to 10 ml of a chitosan solution (cf. Example 1), yield 0.1280 g.

AAAS11-6: 0.500 g SOS-Na₈ in 40 ml distilled water is slowly added through a tip for a pipette to 10 ml of a chitosan solution (cf. Example 1), yield 0.1749 g.

- 10 AAAS12-4: 10 ml of a chitosan solution (cf. Example 1) is dropwise added to 0.150 g SOS-Na₈ in 40 ml distilled water, yield 0.1825 g.

AAAS12-6: 10 ml of a chitosan solution (cf. Example 1) is dropwise added to 0.500 g SOS-Na₈ in 40 ml distilled water, yield 0.1638 g.

AAAS13b-4: a chitosan film is formed by completely drying of a 1% chitosan solution. Then 0.4 g SOS-Na₈ dissolved in 20 ml water is added.

- 15 AAAS13c-3: a film formed by SOS-Na₈ prepared by Method III given above in the reverse order.

AAAS15-1 and AAAS15-3: A chitosan solution (cf. Example 1) is poured into a petri dish and a chitosan film is obtained after evaporation of the solvent. Then a solution of SOS-Na₈ is added (i.e. equivalent to Method III given above)

- 20 Various other preparations have been performed, e.g. chitosan has been employed in form of its glutamate or its hydrochloride. However, the initial results indicate that the viscosity of the reaction solution obtained by employment of these salts is too low to obtain a spheric product. Employment of the salts may therefore involve use of a viscosity adjusting agent if a spheric product is desired.

- 25 One batch (AAAS-5-2) was chosen for further characterisation. The batch was made after method I and the concentration of sodium sucrose octasulfate employed was 5 mg/ml. The characterization of the combination obtained is described in Example 6.

The following method IV has been employed for the preparation of:

AAAS 62*, 64, 67, 70, 72, 74, 75 and 76 (Seacure CL 313, batch 7010301)

AAAS 63*, 65, 69, 71 (Protasan CL 210, batch 6061703)

AAAS 66 (0.5% w/v chitosan, Seacure CL 313, batch 7010301)

- AAAS 79 contains the batches AAAS 64, 67, 70, 72, 74, 75 and 76 (prepared from Seacure CL
5 313); results from elemental analysis (mean of two determinations): S: 11.3, N: 3.3 (including
about 20% w/w of free SOS determined as described below).

AAAS 82 contains the batches AAAS 65, 69, 71, 76 and 77 (prepared from Protasan CL 210);
results from elemental analysis (mean of two determinations): S: 11.8, N: 3.2

AAAS 85-86 (Protasan CL 210, batch 7032401)

- 10 AAAS 102-113 (subjected to the washing procedure described below; Protasan CL 210, batch
7032401); pooled as AAAS 114; results from elemental analysis of the 11 individual batches AAS
102-113 (n=22): S: 10.8 ± 0.29, N: 3.6 ± 0.16 (including free SOS present)

* = prepared from 100 ml 0.1% w/v chitosan solution and 400 ml 1.25% w/v SOS solution

- Method IV 500 ml of chitosan solution (10 mg/ml) is added slowly and dropwise by a pump
15 (Watson-Marlow), rate 10) through a tin (Finn 5-50 µl) to 2000 ml of SOS solution
(12.5 mg/ml) at room temperature. The 500 ml of chitosan solution is added over a
time period of about 3 hours. Small transparent spheres containing the chitosan
solution are formed. The obtained mixture is allowed to react for 4 hours from start
of the experiment. The chitosan solution reacts with SOS on the surface of the
20 drops and small spheres are formed and filtered off using a paper filter.

Washing procedure:

- I The chitosan-SOS formed is subjected to a washing procedure in order to remove
any unbound or loose bound water-soluble SOS. The washing procedure involves
suspending of the chitosan-SOS in about 200 ml of distilled water while stirring.
25 The liquid is filtered off and the material left on the filter is slowly poured over with
approximately 200 ml of water twice. The remaining material is dried in an oven at
60°C for 2 hours. Dried flakes are obtained.

II The chitosan-SOS formed is subjected to a washing procedure in order to remove any unbound or loose bound water-soluble SOS. The washing procedure involves suspending of the chitosan-SOS in about 200 ml of distilled water for 15 min while stirring. The liquid is filtered off and the procedure is repeated once. After filtering, the material left on the filter is slowly poured over with approximately 100 ml of water twice. The remaining material is dried in an oven at 60°C for 2 hours. Dried flakes are obtained.

5 Milling of the chitosan-SOS:

The products obtained are clear and very hard flakes having a pale yellowish colour. 10 These flakes can only with difficulties be pulverized to small particles by means of a mortar. When milling in a ball mill (Centrifugal Ball Mill model S 1000) in 2 x 30 min, a white yellowish powder is obtained which is free-flowing. The mean particle size (AAAS 79 and AAAS 82) is determined to 50-60 µm by use of Coulter Multisizer II and employing 0.9% sodium chloride and 0.1% Tween 80 as an 15 electrolyte solution.

Method IV and Washing procedure II have been employed in the preparation of chitosan-SOS combinations of varying degrees of loading with SOS. Many of the batches mentioned above have a SOS load of about 45% w/w (including free, unbound and loose bound SOS). The combinations are prepared starting from the following ratios of chitosan:SOS (w/w) and with 20 100 g chitosan (Seacure Cl 313) as starting material:

1. Batch BHQ01 1:1 (the resulting product was transparent white drops)
2. Batch BHQ02 1:5 (the resulting product was spheric white drops)
3. 5:1 (only very small yield)
4. Batch BHQ03 2:1 (pale white drops)

25 The solutions used were:

Chitosan solution: A 1% w/v (10 mg/ml) chitosan solution is prepared by slurring 5.0 g chitosan in 250 ml of distilled water followed by adding 250 ml of a 2% w/v solution of acetic acid. The resulting mixture, i.e. a 1% w/v solution of chitosan in 1% w/v acetic acid, is stirred vigorously for 60 min or until dissolution has taken place. Alternatively, the chitosan can be dissolved 30 directly in 1% acetic acid solution.

SOS solution: 1, 2.5, 5 and 25 g sucrose octasulfate sodium salt (SOS) is added to 2000 ml deionized water containing 0.5, 1.25, 2.5 and 12.5 mg/ml of SOS.

After isolation and washing of the product obtained it is dried and the yield is determined. Elemental analysis is also performed and the results are the following:

Results

	Batch	Chitosan: Na ₈ -SOS	Nitrogen (%)	Sulphur (%)	mol sulphur	mol SOS	g SOS per 100 g composition
5	BHQ03	2:1 ^a	4,9	6,6	0,206	0,026	25,3
	BHQ01	1:1 ^b	4,6	8,8	0,275	0,034	33,1
	BHQ02	1:5 ^c	4,3	9,3	0,291	0,036	35,1

^a 2 mg chitosan/ml to 1 mg Na₈-SOS/ml

^b 2 mg chitosan/ml to 2 mg Na₈-SOS/ml

10 ^c 2 mg chitosan/ml to 10 mg Na₈-SOS/ml

The results show that the load increases with increasing concentrations of SOS solution employed. Furthermore, it seems as if the maximal loading of chitosan with SOS is almost reached.

15 The products obtained were subjected to testing for free, unbound or loose bound SOS. 20 ml of distilled water is added to 100 mg of the product obtained (concentration 5 mg/ml). The mixture is magnetically stirred at room temperature. At time t = 1 min, 30 min, 2 hours, 24 hours a 2 ml sample is withdrawn and filtered. The filtrate is analyzed for SOS. None of the samples analyzed from batches HQ01-03 contained any free SOS.

Elemental analysis of the products obtained has shown that

- 20 * the stoichiometric composition of the product (the combination) is independent of the batch size
- * the product obtained can be produced in a reproducible manner
- * a product made according to the general method described above (AAAS 79 and AAAS 82; method IV without any washing procedure) contains about 12% sulfur and 3% nitrogen
- 25 corresponding to about 45% SOS and about 55% chitosan (including loose bound and unbound SOS)
- * the use of different qualities of chitosan with the same degree of deacetylation corresponding to about 84% (Protasan CL 210 and Seacure CL 313) as starting materials

- does not result in a significant difference in the products obtained with respect to the content of S and N
- * employment of the washing procedure described above is an advantage in order to remove any loose bound or unbound SOS from the products (compare the results from the elemental analysis of AAAS 114 (washed) and AAAS 82 (unwashed)).

Other conditions than the ones given above may of course influence the composition and the yield of the combination obtained such as, e.g., the method of preparation (e.g. the mixing process, the mixing process and the order in which the components is added, the ionic strength prevailing in the reaction mixture, the viscosity, the kind of solvent chosen for the reactants, any addition of accelerators, the ratio of SOS and chitosan, the pH prevailing in the reaction mixture, reaction time, rotation speed, e.g. from 0-1000 rpm, homogenisation of the reaction mixture, heating/cooling of the reaction mixture, employment of ultrasonic treatment (cf. Example 5), the starting materials (chitosan, chitosan glutamate, chitosan chloride, chitosan lactate, sucroseoctasulfate as hydrate or other solvates or as a sodium, potassium or another salt) and the concentration thereof (the concentration of chitosan may be varied from about 0.1% to about 20% w/v and the concentration of SOS may be varied from about 0.1% to about 50% w/v), the precipitation method, any purification step etc.). Investigations are still ongoing with regard to testing relevant process parameters.

EXAMPLE 5

- 20 Preparation of a chitosan-sodium sucroseoctasulfate film
(see also method III described in Example 4)

10 ml of a 1% w/w solution of chitosan (Sigma) in 1% w/w acetic acid is poured into a petri dish (9 cm in diameter) and the solvent is evaporated in air at room temperature for at least 4 hours or, alternatively, the solvent is evaporated in an oven at 40-60°C resulting in a chitosan film.

25 Then 20 ml of an aqueous solution of sodium sucrose octasulfate (SOS-Na₈) is poured on the dried chitosan and left at ambient temperature. After 24 hrs a milky precipitate is formed and a soft film is obtained.

A chitosan-sodium sucroseoctasulfate film can also be obtained starting from pouring a sodium sucroseoctasulfate solution into a petri dish and then adding a chitosan solution thereto.

EXAMPLE 6**Characterization of a chitosan-sodium sucrose octasulfate combination (batch AAAS-5-2 and batch AAAS 82)****A. Differential Scanning Calorimetry**

5 The following materials were subjected to Differential Scanning Calorimetry:

- * Batch AAAS-5-2 (chitosan-SOS combination)
- * Sodium sucroseoctasulfate from BM Research
- * Chitosan (C-3646) from SIGMA
- * A physical blend of 25 mg of sodium sucroseoctasulfate and 5 mg of chitosan (5:1) (The
10 ratio between SOS-Na₈ and chitosan has proved not to have any significant influence on
the result)

The thermograms obtained are enclosed (see Fig. 1-4) and they show that

- i) chitosan decomposes at about 230°C;
- ii) sodium sucroseoctasulfate has a sharp endotherm at about 100°C probably indicating a
15 melting of sodium sucroseoctasulfate hydrate. The content of water determined by Karl Fisher gives 15-16 % water in the compound corresponding to about 12 water of hydration per molecule. Thus, the starting material for all experiments given herein has been sodium sucroseoctasulfate hydrate.
- iii) sodium sucrose octasulfate decomposes at about 150°C;
- iv) the physical blend of chitosan and sodium sucroseoctasulfate shows a sharp endotherm at
20 about 100°C, a decomposition of sucrose octasulfate at about 150°C and a decomposition of chitosan of about 230°C;
- v) the AAAS-5-2, i.e. the chitosan-sodium sucrose octasulfate combination, has none of the
25 above-mentioned characteristics, i.e. the thermogram for the combination does not have any endotherm at 100°C nor shows it any decomposition of sodium sucrose octasulfate and chitosan at about 150°C and about 230°C, respectively.

In conclusion, the AAAS-5-2, i.e. the chitosan-sodium sucrose octasulfate combination, shows thermal properties which are different from the individual materials as well as from a physical blend of the individual materials, i.e. the chitosan-sodium sucroseoctasulfate combination is not
30 a mere physical blend of the materials.

B. Solubility

The solubility of batch AAAS-5-2 was investigated in various media and compared with the solubility of the individual materials alone.

The solubility was determined visually by adding 5 mg of batch AAAS-5-2 (pulverized in a mortar) to 20 ml of the medium under investigation. The mixture was then heated to about 5 50°C on a ultrasonic bath for 30 min.

The solubility of the chitosan-sodium sucroseoctasulfate combination (pulverized in a mortar) was investigated in the following media:

1. hydrochloric acid (0.1M and 1.0M, respectively)
2. hydrochloric acid (0.1M and 1.0M, respectively) with addition of 10% w/v sodium chloride
10 solution (increasing the ionic strength could increase the solubility of the compound if the combination was formed based on a ionic bond between the two reactants)
3. sodium hydroxide (1.0M)
4. sodium hydroxide (1.0M) with addition of various amounts of potassium chloride (see the explanation under 2.)
- 15 5. dimethylsulfoxide (DMSO)
6. 0.6M ammoniumsulfate having a pH adjusted to pH 3.5 and with addition of various amounts of 10% w/v sodium chloride (the medium corresponds to the mobile phase system applicable in the HPLC-assay for the determination of sodium sucrose octasulfate in the monography "Sucralfate" as described in USP 23 (1995) page 1443

- 20 None of the above-mentioned media dissolved the chitosan-sodium sucrose octasulfate combination under the conditions given above (heating at 50°C on a ultrasonic treatment for 30 min). However, all the experiments were run for only 30 min and in the experiments with a relatively high ionic strength it is likely that some of the combination has dissolved. In general, the solubility of the combination in these media seems to be less than 0.025% w/v under the
25 conditions given.

Solubility of sodium sucrose octasulfate in the above-mentioned media: soluble in media 1, 2, 3, 4 and 6 and only sparingly soluble in media 5 (less than 0.025 % w/v, i.e. less than 0.25 mg/ml). SOS-Na₈ is also soluble in 1% w/v acetic acid.

Solubility of chitosan (as base) in the above mentioned media:

- 30 As mentioned above, chitosan is not soluble at pH above 7.0; therefore, to avoid precipitation, the pH must be maintained at pH 6.0 or below.

Generally the solubility of chitosan in inorganic acids is as follows:

HCl/HNO₃: Chitosan is soluble in a 0.15-1.1 % solution of the acid but insoluble in a 10 % solution of the acid.

H₂SO₄: not soluble at any concentration.

- 5 H₃PO₄: Only slightly soluble in a 0.5% solution of the acid.

Solubility of chitosan in the above mentioned media and under the conditions given: not soluble in media 1 (0.1 M and 1.0 M HCl), 2 (0.1 M and 1.0 M HCl with sodium chloride), 3, 4, 5 and 6.

In media 1 and 2 chitosan is only sparingly soluble after 30 min on the ultrasonic bath.

- 10 However, additional experiments have indicated that chitosan is soluble in hydrochloric acid but the solubility rate is very slow. Furthermore, chitosan is soluble in weak organic acid such as in 1% w/v acetic acid.

A preferred method to make a 1 % chitosan solution is to slurry the chitosan in water and then add the solution of the desired organic acid (normally acetic acid). This mixture should be stirred vigorously for 60 minutes or until complete solubility is realized. Alternatively, chitosan

- 15 can be dissolved directly by adding chitosan to a prepared 1 % solution of the organic acid.

The solubility may also be determined by trituration. An accurate amount of the compound/material under testing is loaded into a tube and then an accurate volume (e.g. from 10 to 500 µl) of the dissolving medium is added. The mixture is stirred at a constant temperature and after a fixed period of time e.g. from 1 to 24 hours, the mixture is visually

- 20 inspected and if solid particles still are present then an additional volume of the medium is added. This procedure is repeated until dissolution of the compound/material is completed. The solubility is then calculated taking into account the accurate amount of the compound/material tested and the total volume of the medium used for dissolution.

Alternatively, the solubility can be determined at e.g. 22°C using a series of different

- 25 concentration ranges of the combination. The test is run for e.g. 24 hours or longer, if necessary.

Solubility of AAAS 82

The aqueous solubility of chitosan-SOS (AAAS 82) (premilled to a mean particle size of about 50-60 µm) was determined in deionized water and 0.9% w/v sodium chloride solution,

respectively. The solubilities were examined at room temperature (about 22°C) by adding excess

- 30 amounts of the compound to the solvents in tightly closed containers. The mixture were placed in an ultrasonic water bath for about 30 min and then rotated on a mechanical spindel for 2-3 days. It was ensured that saturation equilibrium was established. A concentration range of

chitosan-SOS in the solvents was examined. The solubility was assessed by visually inspection of undissolved particles in the glass containers.

It was found that the aqueous solubility of chitosan-SOS (premilled AAAS 79) in both deionized water and in 0.9% w/v sodium chloride solution, respectively, is less than 0.001 mg/ml (1

5 µg/ml).

Furthermore, the solubility of chitosan-SOS in 1 M aqueous ammoniumchloride (pK_A 9.25) pH 5.0 has been found to be less than 0.01 mg/ml.

In conclusion, SOS-Na₈ and chitosan are dissolved in 1% w/v acetic acid and in weak acid solutions whereas a combination of chitosan-SOS-Na₈ is insoluble in 1% w/v acetic acid.

- 10 10 Furthermore, variation in the composition of chitosan and SOS (e.g. the load of SOS on chitosan) may give rise to a variation with respect to solubility properties and other physico-chemical properties). However, the solubilities of BHQ01, BHQ01 and BHQ03 in demineralized water and 0.9% w/w sodium chloride are less than 0.01 mg/ml using the above-discussed method.
- 15 15 C. Release of sodium sucroseoctasulfate from a chitosan-sodium sucrose octasulfate combination by means of lysozyme

Lysozyme is an enzyme which is present in wounds of mammals and, furthermore, Lysozyme L 6876 from Sigma is able to hydrolyze chitosan to glucosamine residues. The aim of the study was to investigate whether the influence of lysozyme on a chitosan-sodium sucrose octasulfate combination releases sodium sucrose octasulfate from the combination.

The following experiments were performed (please note that these experiments are only preliminary and further experiments are needed in order to have substantial documentation):

1. to 40.0 mg of AAAS-5-2 (i.e. a chitosan-sodium sucroseoctasulfate combination) was added 1.0 ml of 1.0% w/v lysozyme dissolved in distilled water and then diluted to 50.0 ml with
25 25 distilled water
2. to 10.0 mg of chitosan was added 1.0 ml of 1.0% w/v lysozyme dissolved in distilled water and then diluted to 50.0 ml with distilled water
3. to 10.0 mg of sodium sucroseoctasulfate was added 1.0 ml of 1.0% w/v lysozyme dissolved in distilled water and then diluted to 50.0 ml with distilled water

The mixtures (only SOS-Na₈ in experiment 3 above is in the form of a solution) were placed on a shaking table for 2 hours at room temperature. The three tests were examined visually. Only experiment 1 and 3 showed clear solutions, i.e. the materials had reacted/dissolved. The chitosan in experiment 2 had not completely degraded. The result indicate that a reaction has

- 5 taken place in experiment 1 whereas the degradation of chitosan itself was not completed within the test period (2 hours).

The amount of sodium sucrose octasulfate released (from the combination of experiment 1 and from experiment 3, respectively) was determined by employment of the HPLC assay described in USP 23 (1995) page 1443 for sucralfate.

- 10 The results show that about 22.1 % SOS is found in experiment 1 and 82.8 % in exp. 3 (based on the initial concentration of SOS in the solutions). Hydrolysis of chitosan with lysozyme releases SOS but, apparently, lysozyme or the conditions prevailing also seems to have an effect on the SOS in solution. However, these results are only preliminary and further experiments are carried out in order to follow the release of SOS from a combination according to the
15 invention as a function of time and also to investigate whether SOS is degraded by means of enzymes or the experimental conditions prevailing during the experiment. Furthermore, experiments are carried out e.g. i) with enzymes having well-defined enzyme activity, ii) by applying other enzymes (e.g. various glucosidases, proteases, elastase, chitonase) and iii) at conditions where the enzyme used have its optimum activity (e.g.lysozyme-activity is very
20 dependent on the pH of the solution and has its the maximum activity at about pH 5).

D. Elemental analysis of a chitosan-sodium sucroseoctasulfate combination (batch AAAS-5-2 and AAAS19)

AAAS19 was made according to method I (see Example 4) and the concentration of sodium sucrose octasulfate was 12.5 mg/ml.

25 Results:

AAAS5-2: C: 26.5%, H: 4.5%, N: 3%, O: 46.7%, S: 8.5%

AAAS19: C: 27.9%, H: 4.8%, N: 4.2%, O: 46.0%, S: 9.6%

- 30 The content of SOS in batch AAAS5-2 and AAAS19 is calculated to be about 33 % w/w and 37 % w/w, respectively. The elemental analysis can be used to support the results obtained from other experiments concerning determination of the SOS-content in the combination.

E. Determination of pH of chitosan and sucrose octasulfate containing solutions

10 ml of a solution of 1% w/v chitosan in 1% w/w acetic acid was added to 6 different aqueous solution (40 ml of each) of sucrose octasulfate in a concentration range corresponding to 1.25 mg/ml-12.5 mg/ml. After a reaction time of 40 min, the precipitated solid material (combination 5 of chitosan and SOS-Na₈ according to the invention) was filtered off and pH was measured in the filtrate.

3 different solutions of chitosan were employed and the three different types of chitosan described under "Materials" were employed. Thus, totally the pH of 18 filtrates was measured.

Results:

- 10 A solution of chitosan from SIGMA added to the 6 solutions of SOS all gave a pH about 4.4.
A solution of Seacure CL 310 added to the 6 solutions of SOS all gave a pH about 3.5.
A solution of Seacure 110 G added to the 6 solutions all gave a pH about 3.8.

The difference in molecular structure between the three types of chitosan can explain the difference in pH (especially the deacetylation grade of the chitosan may be of importance here).

- 15 Finally, pH was measured in six aqueous solutions of SOS-Na₈ (concentration 1.25 mg/ml, 1.88 mg/ml, 2.50 mg/ml, 3.75 mg/ml, 5.0 mg/ml and 12.5 mg/ml, respectively).

Results:

1.25 mg/ml: pH= 4.2; 1.88 mg/ml: pH= 5.8; 2.50 mg/ml: pH= 6.1
3.75 mg/ml: pH= 6.3; 5.00 mg/ml: pH= 6.6; 12.5 mg/ml: pH= 6.8

- 20 Thus, increasing the concentration of SOS increases the pH of the solution.

F. Characterization by means of IR, solid phase NMR and X-ray diffraction

FTIR: An apparatus 1600 from Perkin-Elmer is employed and a KBr technique is used employing the following procedure:

- Weigh out and pulverize 1 mg of a sample in a mortar. Add 300 mg KBr of 3-4 times (pulverize 25 between the additions of KBr). Place the powder in a container and form it to a capsule. The capsules formed are placed in a capsule-holder in the apparatus and the IR-spectrum is measured.

- NMR: Solid state spectroscopy is employed: About 200-300 mg of sample is placed in a sample tube which subsequently is placed in a probe. The probe is located in a gap in the middle of the cryomagnet. To ensure homogeneity the sample is rotated rapidly so that any inhomogeneity is averaged out. When the magnetic field over the bulk of the sample is homogeneous and well controlled, the spectrum is recorded.

X-ray diffraction: The Debye-Scherrer method is employed: A monochromatic X-ray beam is diffracted by a powder sample. The crystallinities give rise to cones of intensity which are detected by a photographic film wrapped around the circumference of the camera.

G. Other investigations

- 10 Chitosan-SOS combinations were precipitated in 6 different aqueous solutions of SOS (conc. range 1.25 mg/ml-12.5 mg/ml) after method I in Example 5. The content of SOS was then determined in the solutions after precipitation of the combination and filtration. The concentration was measured on the HPLC-system described in the apparatus-section.

The results show that with increasing amount of SOS added the load of SOS in the combination increases. Thus, combinations having different composition with respect to the content of SOS can be obtained by varying the preparation conditions such as, e.g., the concentration of the chitosan solution and the concentration of the SOS solution.

- 15 The results of this experiment do not give a clear picture of the structure of the combination but reveal that some kind of binding forces may be operating. Without being bound to any theory, 20 the chitosan-SOS in the combination may be based on the following structures:

- complex
- ionic binding
- gel structure
- inclusion/incorporation in an net-like or otherwise structure
- 25 adsorption
- inclusion,
- and more unlikely, a covalent binding.

Most likely the combination is held together via ionic binding and, therefore, it is contemplated that the pH of a reaction medium is of importance for the formation/yield/structure of the 30 combination.

EXAMPLE 7**In vivo investigation of the wound healing effect of a chitosan-sodium sucroseoctasulfate combination****A. A clinical study of donor site healing after autologous split skin transplantation**

- 5 The aim of the study is to document a potential benefit of using a combination of chitosan-SOS in the healing of skin wounds.

Twenty patients with preferably two split donor sites receive randomly selected between the two donor sites, traditional wound dressing (control) or traditional wound dressing plus chitosan-SOS combination (test). Wound dressings are changed every two days and time to complete

- 10 healing of the donor site is recorded.

B. A clinical study of treatment of leg ulcers

Leg ulcers are a very important clinical problem demanding large resources in both primary and secondary healthcare service and incapacitating a high number of the elderly people.

- 15 A randomised study of healing of leg ulcers in two parallel patient groups. Patients from a wound treatment clinic with ulcers that is expected to be able to heal, and not to be treated with skin transplant are selected for the study. Leg ulcers receive traditional treatment (control) or traditional treatment plus a chitosan-SOS combination or alternatively, leg ulcers receive placebo (control) or treatment with a combination according to the invention. Primary end point is "ulcer closure rate" calculated after measuring ulcer area weekly. Secondary end points are time to
- 20 healing and overall number of completely healed ulcers in the two study groups.

EXAMPLE 8**Assessment of tissue reactions to different wound dressing in full thickness wound study in pigs**

- Before a study as the one described in the following is started, a dose-finding study is normally carried out in order to establish the effective concentration of the active ingredient. Such studies may be performed according to established guidelines for good clinical practice (GLP) or Investigational New Drug Exemption regulations as described in the description.

The objective of the present study is to assess the tissue reactions in full thickness wounds in pigs after treatment with the following four test samples:

TA-1: chitosan

TA-2: sodium sucrose octasulfate

5 TA-3: a combination of chitosan-sucrose octasulfate according to the invention

TA-4: a physical blend of chitosan and sodium sucrose octasulfate

TA-5: no treatment

As control the following can be used (dependent on the formulation of the test samples used; the test samples may be applied as solid material with or without any addition of excipient(s) or

10 they may be applied in a vehicle): i) Tegaderm®, ii) vehicle corresponding to vehicles employed for the test samples, iii) blank. The substances may also be applied in the form of a gel preparation such as a 2.6% w/w Natrosol 250 HX pharm in distilled water containing the test substance. Gels containing 5-40% w/w chitosan-SOS was prepared by slowly adding the polymer powder to the water phase heated to about 80-90°C. The mixture was stirred until a

15 homogeneous clear colourless solution was obtained. 5-40% w/w of the chitosan-SOS combination (AAAS 82) was added to the gel solution. The mixtures were mixed with a mortar and pestle. A homogeneous grey-beige coloured suspension was obtained. The suspension gel does not sediment during storage for a week at 5°C.

The study will be conducted in accordance with OECD principles of Good Laboratory Practice.

20 Pigs have been chosen as experimental animals because pigs have proven to be a good model for assessment of wound healing in humans.

Five-ten female SPF pigs (body weight of about 25-50 kg) from Ellegaard Forsøgsgrise, Dalmose, Denmark are used. An acclimatization period of 5 days will be allowed.

25 The study will take place in an animal room provided with filtered air at a temperature of 21°C ± 3°C, relative humidity of 55% ± 15% and an air change of 10 times/hour. The room will be illuminated to give a cycle of 12 hours light and 12 hours darkness. Light will be on from 6 a.m. to 6 p.m. The pigs will be housed individually in metal cages.

A commercially available pelleted mini-pig diet, Altromin 9023 from Chr. Petersen A/S, Ringsted, Denmark will be offered (400 g per pig twice daily). Analyses for nutritional components and relevant possible contaminants are performed regularly. Certificates of analysis are retained. A supply of autoclaved hay will be given daily.

Twice daily the pigs will be offered domestic quality drinking water ad libitum. Analyses for relevant possible contaminants are performed regularly. Certificates of analysis are retained.

- On the day of arrival, the animals will be assigned the numbers 1, 2, 3, 4 and 5 by a randomization procedure using a randomization scheme. Each pig will be identified by an individual number tagged to the pinna of one ear. In addition each pig will be ascribed a cage card which identifies the pig by animal number and study number.
- 5

Procedure

Wounding procedure

- On the first day (day 0) the pigs will be anaesthetized with Stressimil® (i.m.) and Hypnodil® (i.v.). Atropin (i.m.) will be given at the same time to prevent salivation. The hairs of the back and flank will be clipped with an electric clipper before washing with soap and water followed by disinfection with 70% ethanol. Before surgery the skin of the back will be rinsed with sterile 0.9% saline.
- 10
- Circular full thickness wounds will be prepared surgically (diameter 20 mm). Ten wounds (5 on each site of the midline) will be prepared on each pig. A schematic illustration of number and localization of wounds are given below:
- 15

Wound numbers

nose

1		6
20	2	7
3		8
4		9
5		10

Tail

Distribution of test articles on wounds:

Pig	No. 1	Pig	No.2	Pig	No. 3	Pig	No. 4	Pig	No.5	
Wound No.	Test article									
5	1	TA-1	1	Control	1	TA-4	1	TA-3	1	TA-2
	2	TA-2	2	TA-1	2	Control	2	TA-4	2	TA-3
	3	TA-3	3	TA-2	3	TA-1	3	Control	3	TA-4
	4	TA-4	4	TA-3	4	TA-2	4	TA-1	4	Control
	5	Control	5	TA-4	5	TA-3	5	TA-2	5	TA-1
10	6	TA-1	6	Control	6	TA-4	6	TA-3	6	TA-2
	7	TA-2	7	TA-1	7	Control	7	TA-4	7	TA-3
	8	TA-3	8	TA-2	8	TA-1	8	Control	8	TA-4
	9	TA-4	9	TA-3	9	TA-2	9	TA-1	9	Control
	10	Control	10	TA-4	10	TA-3	10	TA-2	10	TA-1

15 Wound dressings are as follows:

TA-1, TA-2, TA-3, TA-4 see above, Control = no treatment

Tracing

After wounding on day 0 and on day 3, 6, 10 and 12, each wound will be traced i.e. a drawing of the margin of the wound on a sterile transparent film using a thin pencil. On day 3, 6, 10 and 20 12 the epithelial rim will also be traced.

Treatment

After wounding each of the test dressings will be applied to the wounds. The allocation of treatments is described above. This treatment scheme secures and even distribution of the treatments to the different wound positions spread over the five pigs.

25 After treatment the wound can be covered with Tagaderm and thereafter gauze (4 layers) which will be fixed with 2.5 cm Scanpor Tape. Subsequently, the test area will be covered/fixed with Fizomull. Finally the pig will be dressed in Fixonet elasticated net dressing.

Every day or every second day the applied dressings will be removed and new dressings and drug composition will be applied according to the same procedure used on day 0. The pigs are placed in a hammock in order to facilitate the procedure.

Observations

5 Body weight

The animals will be weighed on arrival, on the day of wounding and on the day of termination of the study.

Daily inspections

10 The animals will be inspected at least once daily for signs of ill health. The dressings will be inspected and any detached dressing reapplied or, if necessary, redressed with the same type of dressing.

All observations and actions will be recorded.

Wound observations

15 Each wound will be observed and evaluated daily. The grade of inflammation and exudation will be evaluated. Special attention will be paid to occurrence of non-degraded test article in the wound (occurrence of visible particles in the wound). The wound edge diameter and the epithelial edge diameter will be measured. Photographs of each wound will be taken daily. All observations will be recorded.

Photos

20 The following photos will be taken:

Overview photo of all the wounds

A close-up photo of each wound separately.

Estimation of wound exudate

Estimating the amount of exudate using a score 0-3.

- 0 = No exudate
- 1 = Small amount
- 2 = Medium amount
- 3 = High amount

5 Estimation of infection/irritation

Estimating erythema and/or oedema in the skin surrounding the wounds.

Tracing of wounds (for Planimetric calculations)

Drawing on the transparent sterile film of the outer margin of the wounds. Additionally the epithelial run will be drawn when appearing.

10 Terminal observations

Twelve days after wounding (day 12) the animals will be anaesthetized by a stunning gun and sacrificed by a cut of the vessels to a forelimb.

At termination each wound will be excised with a full thickness cut and at least a 5 mm margin of normal skin around the wound.

15 Each wound will be attached to a piece of cardboard with one piece of gauze in between and fixed in neutral 4% buffered formaldehyde. Care will be taken in order to secure that animal number, wound number and position can be identified.

Histopathology

20 Two tissue samples (sample 1 and 2) from each wound will be paraffin embedded and sections cut at nominal thickness of 5 µm will be stained with haematoxylin - eosin. The slides will be examined microscopically (slide 1 and 2).

A throughout histopathological evaluation including assessment of re-epithelialization will be performed. The tissue will represent two full transversal (medio-laterally) sections of each wound and surrounding skin.

25 A semiquantitative analysis (no. slight, moderate, marked) of the following cell types; lymphocytes, macrophages, plasma cells and heterophil granulocytes will be included.

Planimetry

At photo copy of each tracing sheet is made and the area of each wound and epithelial rim is determined by video planimetry, using a video camera (Kafpa CF8) and an Image Analysis System Cream (Kem-En-Tec software Systems) version 4.0.

5 EXAMPLE 9**Preparation of a cream containing a composition according to the invention**

1.	Polysorbate 80	5 g
	Cetylan	50 g
	Paraffin oil	50 g
10	Glycerolmonostearate 40-50	60 g
2.	Methylparahydroxybenzoate	1 g
	Glycerol 85%	40 g
	Sorbitol	70 g
	Purified water	724 g
15	3. Chitosan-SOS	100 g

1 is melted together at about 70°C and 2 - which immediately before is heated to boiling and cooled to about 65-70°C - is added under stirring. The mixture is cooled under frequent stirring. 3 is sieved through sieve 125 and mixed in the mixture resulting from 1 and 2.

In the cream mentioned above, the concentration of the combination is 10% w/w. Other 20 concentration can be obtained by adjusting the amount of the combination in the cream.

Other cream bases can also be used such as e.g. Decubal® cream (added from about 0.1% to about 40% w/w chitosan-SOS).

EXAMPLE 10**Preparation of a gel - 1**

25 0.5% w/v methyl cellulose
1% w/v chitosan

2% w/w chitosan-SOS combination (pulverized)

2% w/v glycerol

optionally a suitable preservative in an appropriate concentration
up to 100% w/v purified water, adjusted to pH 5.6

- 5 200 ml chitosan (2% w/v) was mixed with 100 ml of methyl cellulose (2% w/v) and then 8 ml of glycerol was added under stirring. 8 g chitosan-SOS combination was dispersed in the highly viscous solution and the volume adjusted to 400 ml with purified water.

EXAMPLE 11

Preparation of a gel - 2

10 10 g Protasan CL 210

2 g methyl cellulose

200 ml purified water, pH 5.5

10 g chitosan-SOS combination (premilled)

0.2 g methylparahydroxybenzoate

- 15 The methylparahydroxybenzoate was dissolved in 190 ml water and then Protasan and methyl cellulose was added. The mixture was shaken for 4 hours at a shaking table at room temperature to obtain a homogeneous viscous solution. The chitosan-SOS combination (pulverized) was dispersed in the viscous phase.

- 20 Other gels have also been prepared containing chitosan-SOS combination in various concentrations (1-5% w/w). The gels are based on the following polymers:

Metolose 90SH-80000 (hydroxypropylmethylcellulose), Syntapharm

Metolose 90SH-15000 (hydroxypropylmethylcellulose), Syntapharm

Natrosol 250 HHX pharm (hydroxethylcellulose), Aqualon

Natrosol 250 HX pharm (hydroxethylcellulose), Aqualon

- 25 With respect to the above-mentioned polymers suitable gels having an appropriate viscosity have been obtained when the concentration of the polymer is in a range of about 2-3% w/w. In general chitosan, SOS, a chitosan-SOS combination and a physical blend of chitosan and SOS,

respectively, is/are added in a concentration range of about 1-10% w/w. The following results have been obtained:

Chitosan-SOS: suitable suspension gels are formed; up to about 40% w/w of chitosan-SOS can easily be incorporated

5 Chitosan: increases the viscosity of the gel, but a suitable gel can be prepared by adjusting the concentration of chitosan and polymer

SOS: In concentrations above 3-5% w/w of SOS the gel separates

Chitosan + SOS (blend): Most likely chitosan-SOS is formed in an uncontrollable manner. Precipitation of chitosan-SOS has been observed. The gels obtained were not suitable for
10 medicinal application purposes as they were inhomogeneous.

Other relevant materials for gels are, e.g., carboxymethylcellulose sodium, pectin, gelatin, carboxymethylcellulose and hydroxyethylcellulose (suitable if growth factors are added), and alginate pH 7 containing hyaluronate.

EXAMPLE 12

15 **Preparation of an ointment**

An ointment is prepared containing the following constituents:

95 g polyethylene (DLS - Danish Drug Standards)

855 g paraffin oil

50 g chitosan-SOS

20 Polyethylene is dissolved in the paraffin oil by heating to 110°C under stirring and the solution is cooled to about 90°C (under stirring). Then the mixture is cooled with a speed of about 2°C/min under stirring (ointment basis). Chitosan-SOS (premilled) is sieved through a sieve 125 immediately before the combination is dispersed in the ointment basis. A white semi-transparent ointment is obtained.

EXAMPLE 13**Preparation of an ointment**

An ointment is prepared containing the following constituents:

- 0.5 % w/v methyl cellulose
5 1.0 % w/v chitosan-SOS combination (pulverized)
2.0% w/v glycerol
water, pH adjustment to about pH 5.6

The ointment is made by mixing 100 ml of an aqueous dispersion of the chitosan-SOS combination (2% w/v) in a mixer with 50 ml methyl cellulose (2% w/v) 50 ml of distilled water 10 is added into the mixer. Finally, 4 ml of glycerol is added under continued stirring. The ointment obtained has a highly viscous consistency.

EXAMPLE 14**Preparation of powders for application e.g. to wounds**

A:

- 15 20 g chitosan-SOS combination (very fine powder)
80 g lactose

The powder was prepared by mixing the chitosan-SOS combination with the lactose using a mortar. The powder can directly be applied to the damaged tissue or it may be covered by a dressing or adhesive.

20 B 1:

- 40% w/w chitosan-SOS combination (very fine powder)
60% w/w sodium alginate

B 2:

- 20% w/w chitosan-SOS combination (very fine powder)
25 80% w/w sodium alginate

C:

- 20% w/w chitosan-SOS combination (very fine powder)

80% w/w collagen

D:

30% w/w chitosan-SOS combination (very fine powder)

70% w/w lactose

- 5 The powders B-D were prepared in an analogues manner to the method described under A. The powders may also be covered by a dressing or an adhesive.

EXAMPLE 15

Preparation of a suspension spray

	Chitosan-SOS combination	100 mg
10	Glucose	475 mg
	EDTA disodium	1 mg
	Microcrystalline cellulose	125 mg
	Polysorbate 80	2.5 mg
	Potassium sorbate	12 mg
15	HCl/NaOH to adjust pH	
	Purified water	ad 5 ml

EXAMPLE 16

Preparation of a topical suspension

	Chitosan-SOS	5.00% w/w
20	Water	76.97% w/w
	Isopropyl alcohol	10.00% w/w
	Polysorbate 80	5.00% w/w
	Hydroxypropylcellulose	1.50% w/w
	Xanthan gum	1.50% w/w
25	Phosphoric acid	0.03% w/w

EXAMPLE 17**Preparation of a cream****A:**

	Arlacel 1689	4.0% w/w
5	Paraffin oil DAB 9	7.0% w/w
	Arlamol E	3.0% w/w
	Chitosan-SOS	25.0% w/w
	Magnesium stearate	1.0% w/w
	Tocopheryl acetate	
10	(α -tocopherol acetate)	2.0% w/w

B:

	Glycerol	3.0% w/w
	Pantheol	1.0% w/w
	Magnesium sulphate	0.7% w/w
15	Preservatives	q.s.
	Purified water	ad 100

Heat A and B separately to 75°C. Add B slowly to A under stirring. Homogenize the mixture intensively. Allow to cool to 25°C with continued stirring.

CLAIMS

1. A combination of a first compound of an oligo- or polysaccharide containing aminosugar units and a second compound of a sulfated mono-, di- or oligosaccharide which - when subjected to suitable tests - at least fulfils one of the following criteria:
 - 5 i) a thermogram obtained by subjecting the combination to Differential Scanning Calorimetry is significant different from thermograms obtained by subjecting the first compound, the second compound and a physical blend thereof, respectively, to Differential Scanning Calorimetry,
 - 10 ii) an IR-spectrum of the combination is different from the spectre of the individual first and second compounds and from a physical blend of the individual first and second compounds,
 - 15 iii) an NMR-spectrum of the combination is different from the spectre of the individual first and second compounds and from a physical blend of the individual first and second compounds,
 - 20 iv) an X-ray diffraction spectrum of the combination is different from the spectre of the individual first and second compounds and from a physical blend of the individual first and second compounds,
 - v) the combination has a solubility in 1% acetic acid at room temperature of at the most about 5% w/w such as, e.g., at the most about 4%, 3%, 2%, 1%, 0.5%, 0.25%, 0.1%, 0.05%, 0.01%, 0.005% or 0.001% w/w, and
 - 25 vi) the combination has a solubility in water at room temperature of at the most about 5% w/w such as, e.g., at the most about 4%, 3%, 2%, 1%, 0.5%, 0.25%, 0.1%, 0.05%, 0.01%, 0.005% or 0.001% w/w.
2. A combination according to claim 1, wherein an aminosugar unit is a sugar unit wherein at least one of the hydroxy groups available of the sugar unit has been substituted by an amino group or an alkanoylated amino group such as an acetylated amino group.
3. A combination according to claim 1 or 2, wherein at the most 30% of the aminosugar units are alkanoylated.

4. A combination according to claim 3, wherein at the most 25% such as, e.g., at the most 10% or 5% of the aminosugar units are alkanoylated.
5. A combination according to any of the preceding claims, wherein the first compound has a degree of amination in the range of 0.1-4, such as in the range of 0.1-4 or 0.1-2, in particular in the range of 0.5-1.5 such as about 1 and the degree of amination being defined as the average number of amino groups or alkanoylated amino groups per saccharide unit.
6. A combination according to claim 5, wherein the first compound has a degree of amination of about 1 such as e.g., a first compound, wherein each sugar unit contains an amine or an alkanoylated amine group.
- 10 7. A combination according to any of the preceding claims, wherein the individual sugar units are the same or different and are hexoses selected from the group consisting of glucose, mannose, galactose, xylose and ribose.
8. A combination according to any of the preceding claims, wherein the first compound is selected from the group consisting of chitosan, chitosans obtained by deacetylated of chitin to various degrees of deacetylation, chitosan derivatives, glycosaminoglycans including chondroitin, chondroitin sulfate, hyaluronic acid, dermatan sulfate and keratan sulfate; aminated dextrans including DEAE-dextran; aminated starch, aminated glycogen, aminated cellulose, aminated pectin, heparin, and salts, complexes, derivatives and mixtures thereof.
- 15 9. A combination according to any of the preceding claims, wherein the combination stabilises and/or stimulates fibroblast growth factors (FGF) in vitro when tested as described herein.
10. A combination according to any of the preceding claims, wherein the combination stimulates the formation of collagen when tested as described herein.
11. A combination according to any of the preceding claims, wherein the combination has a healing effect on wounds in vitro when tested as described herein.
- 25 12. A combination according to any of the preceding claims, wherein the second compound is a polysulfated mono-, di- or oligosaccharide.
13. A combination according to claim 12, wherein the saccharide is a disaccharide.

14. A combination according to claim 13, wherein the disaccharide is sucrose, a sucrose derivative or a complex or salt thereof.
15. A combination according to claim 14, wherein the sucrose is at least tetrasulfated.
16. A combination according to claim 15, wherein the sucrose is sucrose octasulfate or a salt or a complex thereof including the sodium salt thereof.
5
17. A combination according to claim 16, wherein the content of sucrose octasulfate in the combination is in a range of 0.01-95% w/w such as, e.g., in a range of about 20-90% w/w, 25-80% w/w, 30-70% w/w or 30-60% w/w based on the weight of the total combination.
18. A combination according to any of the preceding claims, wherein the first compound is a
10 chitosan.
19. A combination according to claim 18, wherein the chitosan has a molecular weight in a range of about 3,000 to about 1,500,000 daltons.
20. A combination according to claim 18, wherein the chitosan has a degree of deacetylation of at the most 100% such as at the most 99%, 95%, 90%, 85%, or 80%.
- 15 21. A combination according to claim 18, wherein the chitosan has a degree of deacetylation in a range of about 10-90% such as about 20-85%, about 30-80%, about 40-75%, about 50-75%, about 60-85%, about 75-85%, or about 80-90%.
22. A combination according to claim 1, wherein the first compound is a chitosan having a
20 molecular weight in a range from about 5,000 to about 100,000 dalton and the second compound is a sucrose octasulfate.
23. A combination according to any of the preceding claims, wherein the combination is a complex formed between the first and the second compound.
24. A combination according to any of claims 1-23, wherein the combination is a salt formed between the first and the second compound.
- 25 25. A combination according to any of claims 1-23, wherein the combination is in the form of a polymeric network.

26. A combination according to any of claims 1-23, wherein the combination is formed by establishment of an ionic bonding between the first and the second compound.
27. A combination according to any of claims 1-23, wherein the first and the second compound in the combination is held together by establishment of an ionic bonding between the first and the second compound and an inclusion of the second compound in the first compound.
- 5 28. Use of a combination according to any of the preceding claims as a medicament.
29. Use of a combination according to any of claims 1-27 for the preparation of a pharmaceutical composition for the treatment of inflammatory conditions.
30. Use of a combination according to any of claims 1-27 for the preparation of a pharmaceutical composition for accelerating tissue repair.
- 10 31 Use of a combination according to any of claims 1-27 for the preparation of a pharmaceutical composition for the treatment of any sucrose octasulfate demanding condition.
32. A pharmaceutical composition comprising a combination according to any of claims 1-27 and at least one pharmaceutically acceptable excipient.
- 15 33. A pharmaceutical composition according to claim 32, wherein the combination is in a form selected from the group consisting of complexes, salts, polymeric networks, ionic bond materials, inclusions, or mixtures thereof.
34. A pharmaceutical composition according to claim 32 or 33 in a form which is suitable for topical application.
- 20 35. A pharmaceutical composition according to claim 32 or 33 in a form which is suitable for mucosal application.
36. A pharmaceutical composition according to claim 32 or 33 in a form which is suitable for application to damaged tissues.
- 25 37. A pharmaceutical composition according to any of claims 32-36 further comprising an active substance selected from the group consisting of antiviral substances, antibiotic substances, antifungal substances, hormones, growth factors, and mixtures thereof.

38. A method for the preparation of a combination according to any of claims 1-27 comprising the steps of:

- i) dissolving a first compound of an oligo- or polysaccharide containing aminosugar units in a suitable aqueous medium,
- 5 ii) dissolving a second compound of a sulfated mono-, di- or oligosaccharide in a suitable aqueous medium,
- iii) mixing the solutions obtained in steps i) and ii), and
- iv) isolating the thus formed combination.

39. A method for stabilizing a tissue repair agent in a injured tissue comprising applying a 10 combination according to any of claims 1-27 on the injured tissue.

40. A combination of a first compound of an oligo- or polysaccharide containing aminosugar units and a second compound of an active drug substance selected from the group consisting of:

Antiinflammatory agents/analgesics including: salicylic acid, 5-aminosalicylic acid, acetylsalicylic acid (aspirin), paracetamol, active drug substances classified as Non-Steroidal Anti-inflammatory Drugs (NSAIDs), aceclofenac, acetaminophen, acetylsalicylic acid, 5-adenosylmethionine, alminoprofen, benoxaprofen, bermoprofen, bromfenac, bucloxic acid, bumadizum, butibufen, carprofen, cinmetacin, clometacin, clomixin, clopirac, ibuprofen, ibufenac, indomethacin, indoprofen, isofezolac, isoxepac, diclofenac, diflunisal, dipropanetadol, enfenamic acid, enoxolone, etodolac, fenbufen, fencloxic acid, fentiazac, flufenamic acid, flunixil, flunoxaprofen, flurbiprofen, fosfosal, gentisic acid, ketorolac, ketoprofen, ionazolac, loxoprofen, mefenamic acid, nadifloxacin, nalidixic acid, naproxen, oxaprozin, pirazolac, pirprofen, pranoprofen, protizinic acid, salicylamide O-acetic acid, salicylsulfuric acid, salsalate, suprofen, tiaprofenic acid, tolmetin, tropesin, ximoprofen, zaltoprofen, zomepirac, and morphine (bearing a phenol group); penicillins including: amdinocillin, amdinocillin pivoxil, aspoxicillin, azidocillin, benzylpenicillin sodium, benzylpenicillin acid, carindacillin, carpetimycins, carbenicillin, benzylpenicillin, benzylpenicillinprocain, phenoxyethylpenicillin, dicloxacillin, cloxacillin, flucloxacillin, meticillin, amoxicillin, ampicillin, bacampicillin, pivampicillin, piperacillin, mecillinam, hetacillin, oxacillin, panipenem, penicillin G Benethamine, penicillin G Benzathine, penicillin N, penicillin O, piperacillin, pivcefalexin, propicillin, quinacillin, and ritipenem; antibiotics including: 30 acediasulfone, alclofenac, aztreonam, benzyl isothiocyanate, benzoylpas, carumonam, cefaclor, cefadroxil, cefamandole, cefatrizine, cefazedone, cefazolin, cefbuperazone, cefcapene pivoxil,

cefclidin, cephalexin, cinoxacin, ciprofloxacin, enoxacin, enrofloxacin, flomoxef, flumequine, difloxacin, fusidic acid, grepafloxacin, lornefloxacin, lymecycline, merbromin, mupirocin, nifuroquine, ofloxacin, opiniazide, pefloxacin, phthalylsulfathiazole, pipemidic acid, pyrithione, rosoxacin, rufloxacin, salazosulfadimidine, succinylsulfathiazole, succisulfone, tigemonam, and 5 trovalfloxacin; antihypertensive agents including: alacepril, candesartan, carmoxirole, cilazapril, delapril, eprosartan, fosinopril, lisinopril, moevitipril, perindopril, ramipril, and valsartan; antirheumatics including: allocupreide sodium, bucillamine, clobuzarit, lobenzanit; thyroid inhibitors including: 3,5-diiodotyrosine, and tiratricol (antihypothyroid); antiparkinsonians including: Droxidopa; anesthetics (topical) including: Ecgonine; antineoplastic agents including: 10 chlorambucil, denopterin, eflomithine, lonidamine, mycophenolic acid, podophyllic acid, streptonigrin, ubenimex, and 5-fluoruracil; agents for treatment of diabetic neuropathy including: Epalrestat; uricosuric agents including: Ethebenicid; diuretics including: acefulline, bemetanid, mercumallylic acid, mersalyl, piretanide, probenecid, and ticrynafen; choleric agents including: Cholic acid, clanobutin, cyclobutyrol, dehydrocholic acid, fencibutirol, and 15 florantyrone; antihistamines including: bietanantine, cetirizine, fexofenadine, acrivastine, and tranilast; antispasmodic/choleretic agents including: Trepibutone; antithrombotic agents including: Dantroban, dndobufen, isbogrel, ozagrel, tirofiban, triflusul; antiasthmatic agents including: Montelukast, and seratrodast; agents for treatment of infertility in mares including: Fluprostenol; vitamins including: Folic acid, and pantothenic acid; antidote to folic acid 20 antagonist, antianemic (folate deficiency) including: Folinic acid; antiarthritic agents including: Diacerein; anticonvulsant agents including: Gabapentin; sclerosing agents including: 2-hexyldecanoic acid; CNS stimulants including: amineptine, hexacyclonate sodium; anthelmintics including: Kainic acid, netobimin, and stibocaptate; antianginal including: Limaprost; ACE inhibitors including: Captopril, benazepril, enalapril, perindopril, trandolapril, moexipril, 25 fosinopril, ramipril, and lisinopril; antihyperlipoproteinemic agents including: acifran, acipimox, ciprofibrate, clinofibrate, clofibrate acid, pravastatin sodium, fluvastatin, gemfibrozil, meglitol, nicotinic acid, and oxiniacic acid; antiamebic agents including: Thiocarbamizine; luteolytic agents including: Tiaprost; antiulcerative agents including: arbaprostil, carbenoxolone, cetraxate, rebamipide, rosaprostol, rotraxate, sofalcone, and trimoprostil; antiviral agents including: statine; 30 antidepressants including: Tianeptine; anticonvulsants including: Tiagabine; mucolytic agents including: Stepronin; antidiabetic agents including: Repaglinide, tolrestat, and zopolrestat; agents for ulcerative colitis/Chrons' disease including: balsalazine, ipsalazine, sulphosalazine, sulfasalazine, mesalamine, olsalazine, and 5-aminosalicylic acid; antiepilectics including: Phenytoin; agents for asthma bronchialis and other broncho-spasms including: Theophyllin; 35 Immunomodulators including: Pidotimod, and procodazole; antifungals including: Ujothion; antiglaucoms including: Unoprostone; Others including: thromboxanes, prostaglandin E2, prostaglandin E1, F2alpha, L-Dopa, hydantoins, allopurinol, diacerein, acetretin, aclatonium napadisilate, actarit, artesunate, benfurodil hemisuccinate, benztrapine mesylate, calcium N-

carbamoylaspartat, calcium 2-ethylbutanoate, capobenic acid, carboprost, chenodiol, clorazepic acid, cromolyn,

which combination - when subjected to suitable tests - at least fulfils one of the following criteria:

- 5 i) a thermogram obtained by subjecting the combination to Differential Scanning Calorimetry is significant different from thermograms obtained by subjecting the first compound, the second compound and a physical blend thereof, respectively, to Differential Scanning Calorimetry,
- 10 ii) an IR-spectrum of the combination is different from the spectre of the individual first and second compounds and from a physical blend of the individual first and second compounds,
- 15 iii) an NMR-spectrum of the combination is different from the spectre of the individual first and second compounds and from a physical blend of the individual first and second compounds,
- iv) an X-ray diffraction spectrum of the combination is different from the spectre of the individual first and second compounds and from a physical blend of the individual first and second compounds.

41. A combination according to claim 40, wherein an aminosugar unit is a sugar unit wherein at least one of the hydroxy groups available of the sugar unit has been substituted by an amino group or an alkanoylated amino group such as an acetylated amino group.

42. A combination according to claim 40 or 41, wherein at the most 30% of the aminosugar units are alkanoylated.

43. A combination according to claim 42, wherein at the most 25% such as, e.g., at the most 10% or 5% of the aminosugar units are alkanoylated.

25 44. A combination according to any of claims 40-43, wherein the first compound has a degree of amination in the range of 0.1-4, such as in the range of 0.1-4 or 0.1-2, in particular in the range of 0.5-1.5 such as about 1 and the degree of amination being defined as the average number of amino groups or alkanoylated amino groups per saccharide unit.

45. A combination according to claim 44, wherein the first compound has a degree of amination of about 1 such as e.g., a first compound, wherein each sugar unit contains an amine or an alkanoylated amine group.

46. A combination according to any of claims 40-45, wherein the individual sugar units are the same or different and are hexoses selected from the group consisting of glucose, mannose, galactose, xylose and ribose.

47. A combination according to any of claims 40-46, wherein the first compound is selected from the group consisting of chitosan, chitosans obtained by deacetylated of chitin to various degrees of deacetylation, chitosan derivatives, glycosaminoglycans including chondroitin, chondroitin sulfate, hyaluronic acid, dermatan sulfate and keratan sulfate; aminated dextrans including DEAE-dextran; aminated starch, aminated glycogen, aminated cellulose, aminated pectin, heparin, and salts, complexes, derivatives and mixtures thereof.

48. A combination according to any of claims 40-47, wherein the first compound is a chitosan.

49. A combination according to claim 48, wherein the chitosan has a molecular weight in a range of about 3,000 to about 1,500,000 daltons.

50. A combination according to claim 48, wherein the chitosan has a degree of deacetylation of at the most 100% such as at the most 99%, 95%, 90%, 85%, or 80%.

51. A combination according to claim 48, wherein the chitosan has a degree of deacetylation in a range of about 10-90% such as about 20-85%, about 30-80%, about 40-75%, about 50-75%, about 60-85%, about 75-85%, or about 80-90%.

52. A combination according to any of claims 40-51, wherein the combination is a complex formed between the first and the second compound.

53. A combination according to any of claims 40-51, wherein the combination is a salt formed between the first and the second compound.

54. A combination according to any of claims 40-51, wherein the combination is in the form of a polymeric network.

55. A combination according to any of claims 40-51, wherein the combination is formed by establishment of an ionic bonding between the first and the second compound.

56. A combination according to any of claims 40-51, wherein the first and the second compound in the combination is held together by establishment of an ionic bonding between the first and the second compound and an inclusion of the second compound in the first compound.

57. Use of a combination according to any of claims 40-56 as a medicament.

5 58. A pharmaceutical composition comprising a combination according to any of claims 40-57 and at least one pharmaceutically acceptable excipient.

59. A method for the preparation of a combination according to any of claims 40-56 comprising the steps of:

- 10 i) dissolving a first compound of an oligo- or polysaccharide containing aminosugar units in a suitable aqueous medium,
- ii) dissolving a second compound of an active drug substance in a suitable aqueous medium,
- iii) mixing the solutions obtained in steps i) and ii), and
- iv) isolating the thus formed combination.

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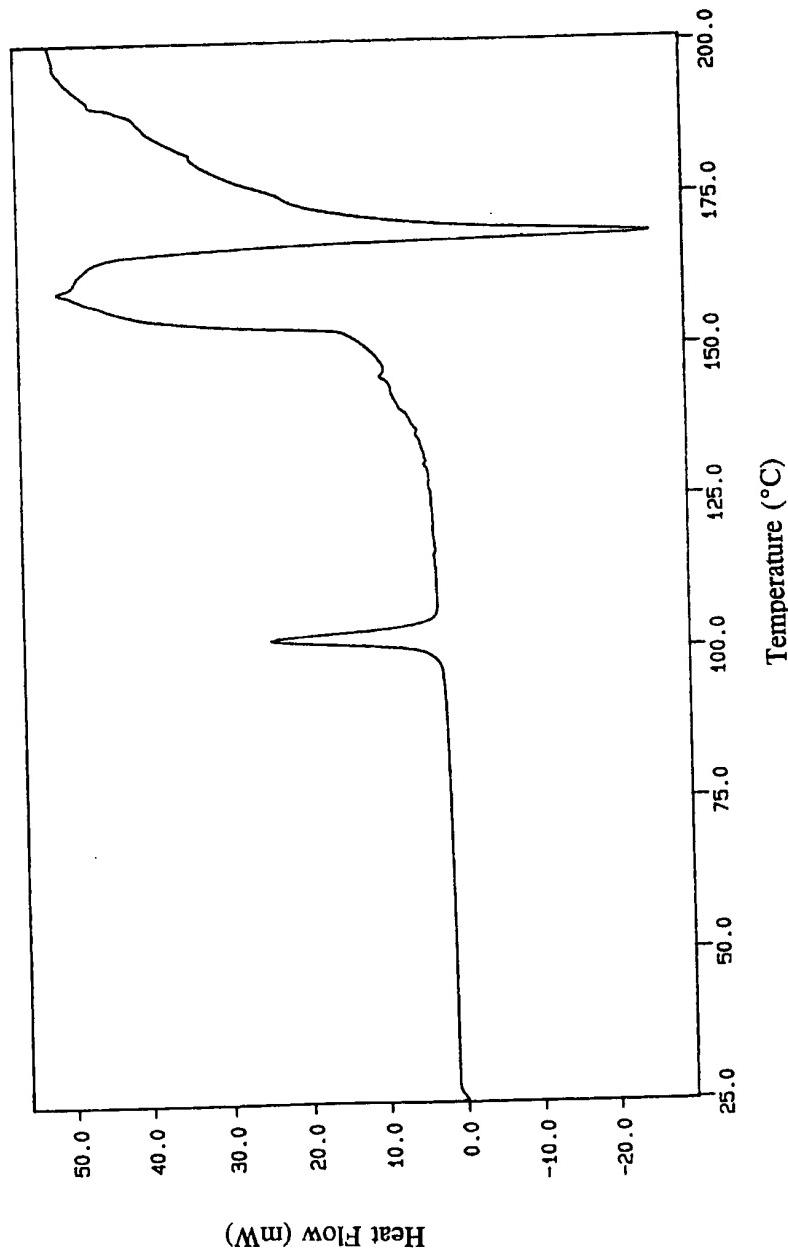


Fig. 1

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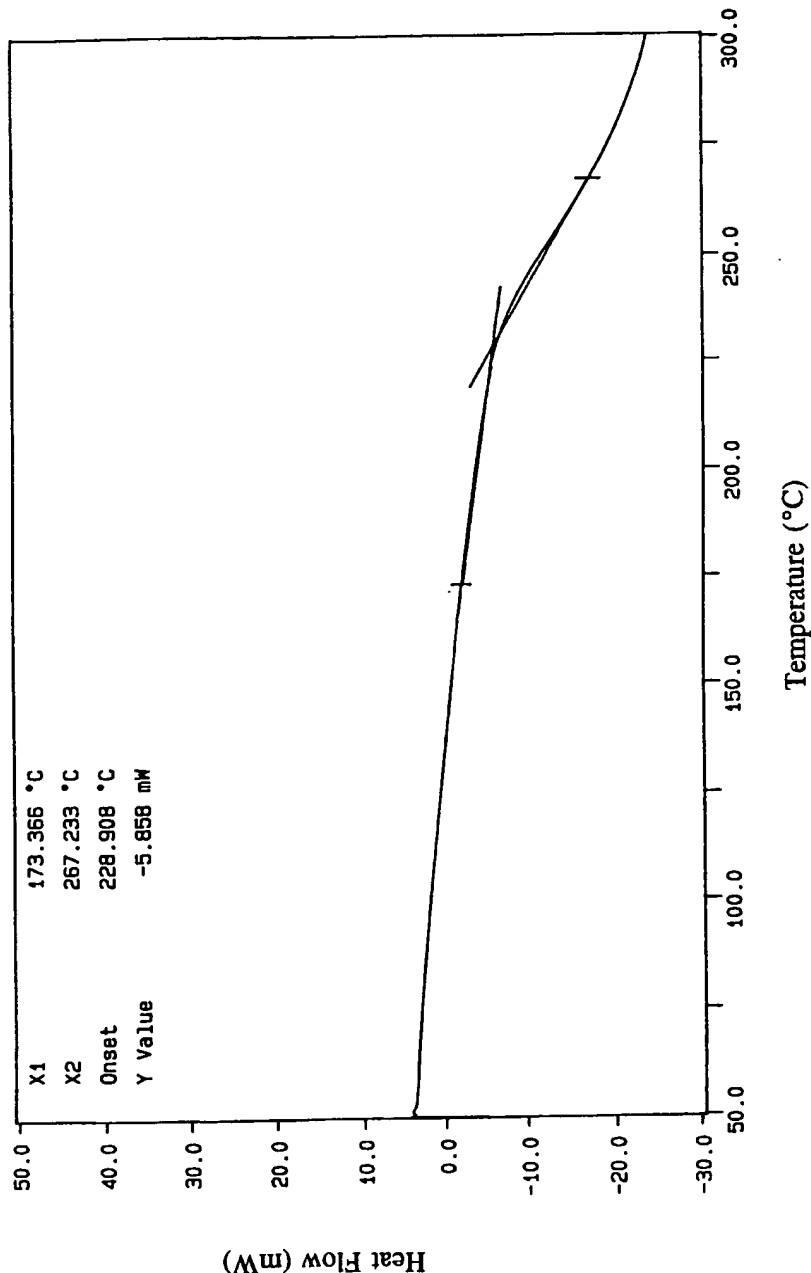
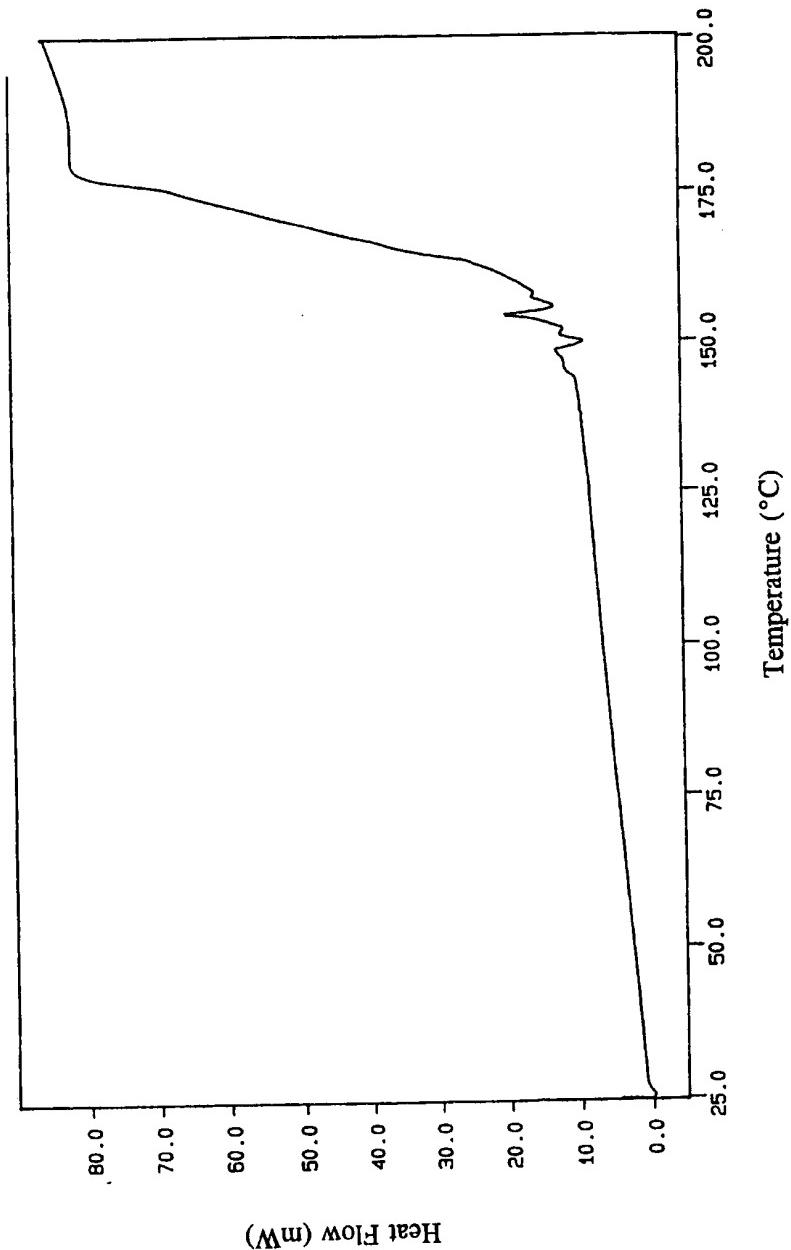


Fig. 2

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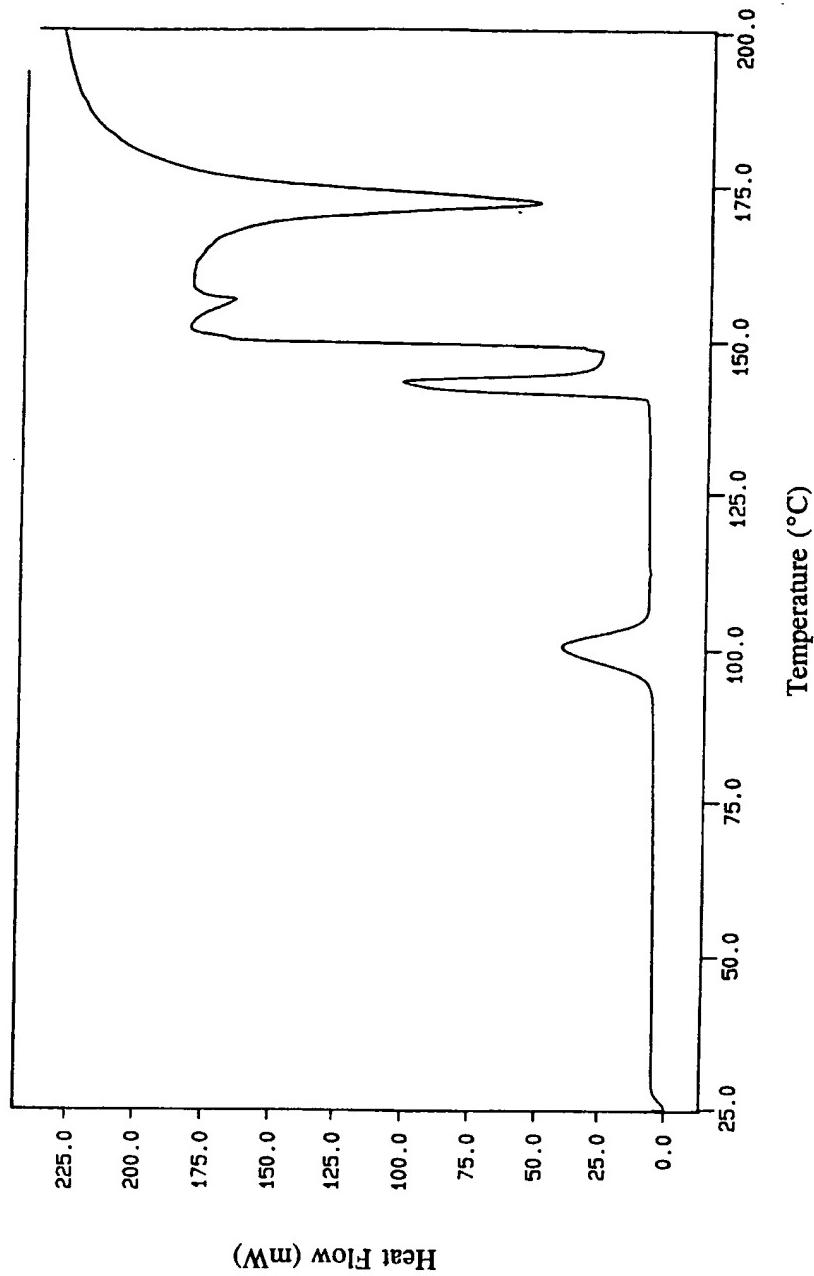
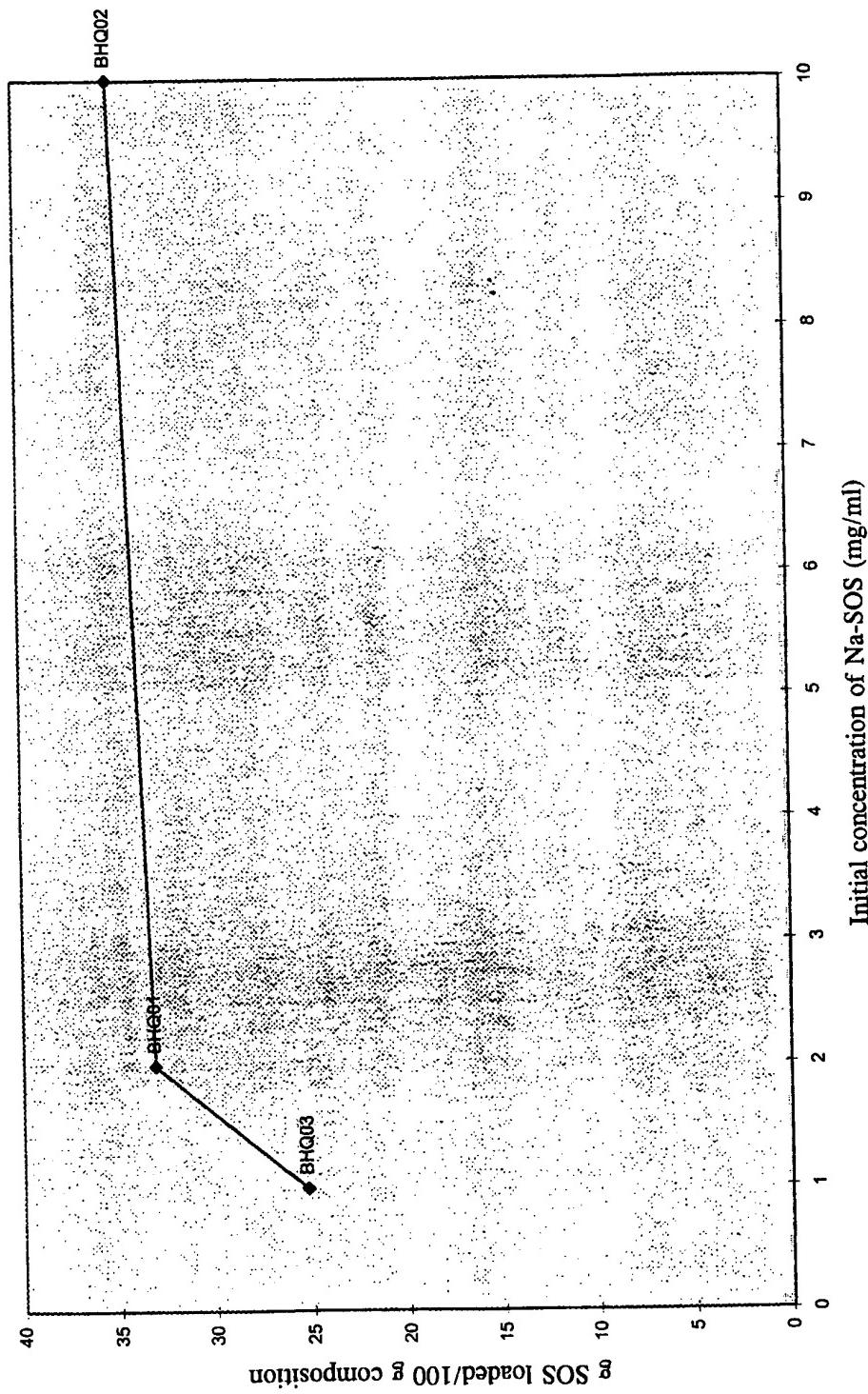


Fig. 4

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Fig. 5

INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 97/00525

A. CLASSIFICATION OF SUBJECT MATTER				
IPC 6	A61K31/70	A61K31/73	A61K9/16	A61K9/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 427 190 A (HOECHST AKTIENGESELLSCHAFT) 15 May 1991 see page 3, line 15 see page 5; examples 3,4	1-12, 18-28, 32,33,38
Y	THO NGUYEN-XUAN ET AL.: "mucoadhesive semi-solid formulations for intraoral use containing sucralfate" EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS, vol. 42, no. 6, April 1996, STUTTGART (DE), pages 133-137, XP000582519 see the whole document	13-17, 29,31, 34-37
	---	13-17, 29,31, 34-36
	---	-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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INTERNATIONAL SEARCH REPORT

Int. Application No.
PCT/DK 97/00525

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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Information on patent family members

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PCT/DK 97/00525

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